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Facultad de Farmacia

Departamento de Química Analítica, Nutrición y Bromatología

Área Nutrición y Bromatología

ESTUDIO DE LOS PARÁMETROS CLAVE IMPLICADOS EN LA MIGRACIÓN DE COMPONENTES DE MATERIALES DESTINADOS AL CONTACTO CON ALIMENTOS

Memoria para optar al grado de doctor

Joaquim Manuel Alves Feiteira Maia

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INFORMA: Que Don Joaquim Manuel Alves Feiteira Maia presenta el trabajo titulado "ESTUDIO DE LOS PARÁMETROS CLAVE IMPLICADOS EN LA MIGRACIÓN DE COMPONENTES DE MATERIALES DESTINADOS AL CONTACTO CON ALIMENTOS" realizado bajo la dirección del Dr. Perfecto Paseiro Losada y del Dr. José Manuel Cruz Freire, en los laboratorios de Bromatología de la Facultad de Farmacia de la Universidad de Santiago de Compostela.

Fdo. Dr. Antonio Moreda Piñeiro

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PERFECTO PASEIRO LOSADA, CATEDRÁTICO DEL ÁREA DE NUTRICIÓN Y BROMATOLOGÍA DE LA FACULTAD DE FARMACIA DE LA UNIVERSIDAD DE SANTIAGO DE COMPOSTELA, Y JOSÉ MANUEL CRUZ FREIRE, PROFESOR TITULAR DEL ÁREA DE INGENIERÍA QUÍMICA DE LA UNIVERSIDAD DE VIGO

AUTORIZAN a Don Joaquim Manuel Alves Feiteira Maia a presentar la Tesis titulada "ESTUDIO DE LOS PARÁMETROS CLAVE IMPLICADOS EN LA MIGRACIÓN DE COMPONENTES DE MATERIALES DESTINADOS AL CONTACTO CON ALIMENTOS" para optar a Grado de Doctor, la cual ha sido realizada bajo nuestra dirección en los laboratorios de Bromatología de la facultad de Farmacia de la Universidad de Santiago de Compostela

Y para que así conste, se expide la presente en Santiago de Compostela en Mayo de 2013.

Fdo. Dr. Perfecto Paseiro Losada Fdo. Dr. José Manuel Cruz Freire

Fdo. Don Joaquim Manuel Alves Feiteira Maia

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"Há um tempo em que é preciso abandonar as roupas usadas, que já tem a forma do nosso corpo, e esquecer os nossos caminhos, que nos levam sempre aos mesmos lugares. É o tempo da travessia: e, se não ousarmos fazê-la, teremos ficado, para sempre, à margem de nós mesmos."

Fernando Pessoa

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Abreviaturas

ρ_F	food density (densidad del alimento)
ρ_P	polymer density (densidad del polímero)
τ	a polymer specific “activation energy” parameter (parámetro especifico del polímero relacionado con la “energía de activación”)
A	contact área between polymer and food (área de contacto entre polímero y alimento)
ACN	acetonitrile (acetonitrilo)
E_A	activation energy (energía de activación)
A_P	parameter related to the polymer that illustrates the basic diffusion behaviour of polymer matrix towards the diffusion of migrants (parámetro que describe el comportamiento de difusión de la matriz polimérica frente a la difusión de los migrantes)
A'_P	an upper-bond athermal term for a given class of polymer (parámetro de difusión relacionado con el polímero e independiente de la temperatura)
BHT	butylated hydroxitoluene (Butilhidroxitolueno)

BP	boiling point (punto de ebulición)
BZP	benzophenone (benzofenona)
CAS	Chemical Abstracts Service
C_P	concentration in polymer (concentración en el polímero)
$c_{P,0}$	initial concentration in polymer (concentración inicial en el polímero)
$c_{P,\infty}$	concentration in polymer when equilibrium is achieved (concentración del migrante en el polímero cuando el sistema llega al equilibrio)
$c_{F,\infty}$	concentration in food when equilibrium is achieved (concentración del migrante en el alimento cuando el sistema llega al equilibrio)
D	diffusion coefficient (coeficiente de difusión)
D^*_P	polymer specific upper-bound diffusion coefficient (límite superior específico del coeficiente de difusión en el polímero)
D_0	pre-exponential factor; theoretical value of D at infinite temperature (factor pre-exponencional; valor teórico de D a temperatura infinita)
DAD	diode array detector (detector de díodos en línea)
D_F	diffusion coefficient within the food (coeficiente de difusión en el alimento)
D_P	diffusion coefficient within the polymer (coeficiente de difusión en el polímero)
DPBD	diphenylbutadiene (difenilbutadieno)
DPP	diphenylphthalate (difenilftalato)

EFSA	European Food Safety Agency (Autoridad Europea de Seguridad Alimentaria)
EPS	expanded polystyrene (poliestireno expandido)
EtOH	ethanol (etanol)
F	food (alimento)
FACET	Flavourings, Additives and food Contact materials Exposure Task
FDA	Food and Drug Administration
FCM	food contact materials (materiales en contacto con alimento)
FLD	flourescence detector (detector de fluorescencia)
GC	gas chromatography (cromatografía gaseosa)
GPPS	general purpose polystyrene (poliestireno de uso general)
HDPE	high density polyethylene (polietileno de alta densidad)
HIPS	high impact polystyrene (poliestireno de alto impacto)
HPLC	high performance liquid chromatography (cromatografía líquida de alta resolución)
ITX	isopropylthioxanthone (Isopropil tioxantona)
$K_{P/F}$	polymer/food partition coefficient (coeficiente de partición entre el plástico y el alimento)
LDPE	low density polyethylene (polietileno de baja densidad)
LLDPE	linear low density polyethylene (polietileno lineal de baja densidad)

Log P (o/w)	octanol-water partition coefficient (coeficiente de partición octanol-agua)
m/z	mass to charge ratio (relación masa/carga)
MeOH	methanol (metanol)
$M_{F,t}$	total amount of migrant that was transferred into the food at t time (cantidad total del migrante que fue transferido al alimento)
$M_{F,\infty}$	total amount of migrant that was transferred into the food when equilibrium was achieved (cantidad total del migrante que fue transferido al alimento cuando el sistema llega al equilibrio)
MP	melting point (punto de fusión)
M_r	relative molecular mass (masa molecular relativa)
MW	molecular weight (peso molecular)
OML	overall oigration limit (limite de migración global)
OM	overall migration (migración global)
P	polymer (polímero)
PA	polyamide (poliamida)
PC	polycarbonate (policarbonato)
PE	polyethylene (polietileno)
PP	polypropylene (polipropileno)
PS	polystyrene (poliestireno)
PVC	polyvinyl chloride (policloruro de vinilo)

q_n	number of roots of the equation tan $q_n = -\alpha \cdot q_n$ (número de raíces de la ecuación tan $q_n = -\alpha \cdot q_n$)
R	ideal gas constant (constante de los gases ideales)
R^2	determination coefficient (coeficiente de determinación)
RMSE	root mean square error (raíz cuadrada del cuadrado medio del error)
RSD	relative standard deviations (Desviación estándar relativa)
SML	Specific Migration Limit (Limite de migración específico)
SML(T)	total specific migration limit (límite de migración específica total)
t	time (tiempo)
T	temperature (temperatura)
TDI	tolerable daily intake (ingesta diaria tolerable)
THF	tetrahydrofuran (tetrahidrofurano)
UV	ultraviolet (ultravioleta)
V_F	food volume (volumen del alimento)
V_P	polymer volume (volumen del polímero)
v/v	volume/volume (volumen/volumen)
W_F	food mass (masa de alimento)
W_P	polymer mass (masa del polímero)
XPS	extruded polystyrene foam (poliestireno extruido)

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Resumen

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Los envases alimentarios son elementos que hacen parte de nuestro día a día y a los cuales estamos acostumbrados. Surgieron y evolucionaron como el resultado de la necesidad del hombre de transportar y conservar los alimentos, y actualmente tienen una importancia fundamental a nivel económico, nutricional y de salud pública. Sin este elemento, el estilo de vida que llevamos sería impensable.

El uso de envases está generalizado y, dependiendo de las necesidades específicas de cada caso, se utilizan de distintos materiales. Actualmente, uno de los más ampliamente empleado y cuyo uso se está incrementado más en los últimos años es el plástico. Esto se debe a diversas ventajas y su adaptabilidad, ya que sus características se pueden modificar, mediante el uso de distintos polímeros y aditivos, en función de las necesidades.

Aunque los envases tengan como función, entre otras, aislar el alimento para evitar contaminación y de esta manera preservar su calidad y seguridad, el propio envase, así como otros objetos que pueden entrar en contacto con el alimento (platos, cubiertos, máquinas de procesado, etc.) pueden interaccionar con el alimento mediante varios fenómenos: sorción, permeación y migración.

Esta tesis se va a centrar en el estudio de la migración, que consiste en la transferencia de sustancias desde el material de envase hacia el alimento, en nuestro caso entre materiales plásticos y alimentos. De los tres fenómenos resultantes de la interacción entre envase y alimento, este es aquel que presenta un mayor riesgo para la salud pública, debido a la naturaleza potencialmente nociva de las sustancias que constituyen el material del envase y debido a los efectos perjudiciales que pueden presentar para la salud del consumidor cuando son ingeridas.

La migración es un proceso físico complejo que depende de varios factores (material de envase, alimento, condiciones de almacenamiento (tiempo y temperatura)) y cuya determinación se puede hacer experimentalmente mediante ensayos de cinéticas de migración. Sin embargo, hacerlo es un trabajo arduo que requiere una gran inversión de tiempo, de mano de obra y es económicamente costoso. Sin embargo, la migración es un proceso físico predecible que puede ser descrito por la ecuación de la Segunda Ley de Difusión de Fick. Se han empleado diferentes soluciones de esta ecuación que permiten determinar la concentración de las sustancias migrantes en el alimento a cada momento. Uno de los autores que presentó un extenso trabajo en esta área fue J. Crank (1975), el cual propuso modelos matemáticos aplicables en diferentes escenarios.

El uso de modelos matemáticos para estimar la migración tiene como ventaja el hecho de que no necesita trabajo experimental, con el consiguiente ahorro económico y de tiempo. Para aplicar estos modelos, hay dos parámetros esenciales: el coeficiente de difusión (D; la cinética del proceso, la velocidad a que el migrante se mueve en la matriz polimérica) y el coeficiente de partición ($K_{P/F}$; el equilibrio termodinámico del proceso de migración, es decir, la proporción entre la concentración del migrante en el polímero y en el alimento cuando se alcanza el estado de equilibrio). Conociendo estos dos datos para una temperatura determinada (solamente D varia con la temperatura; el $K_{P/F}$ no cambia significativamente) es posible determinar la cantidad de migrante presente en el alimento en cualquier momento, sin que sea necesario recurrir a determinaciones experimentales. En caso de que no poseer el D para una temperatura específica, pero si disponer de los valores de D a diferentes temperaturas, es posible estimarlo usando una ecuación de Arrhenius. De esta forma, se comprueba la linealidad del D a lo largo de las temperaturas, y se determinan dos parámetros: la energía de activación y el factor pre-exponencial. Con estos dos valores y en caso que haya linealidad, se puede interpolar el D a la temperatura deseada. Este parámetro también puede ser estimado usando la

ecuación de Piringer. Con esta ecuación, que establece una relación entre el coeficiente de difusión y el tipo de polímero y la masa molecular del migrante, no se determina el D real, pero si un coeficiente de difusión en el polímero sobreestimado (D_P^*). Este valor, al ser sobreestimado, puede ser usado en casos donde es necesario un margen de seguridad.

Los capítulos 2, 3, 4, 5 y 6 tienen como objetivo principal la determinación de los parámetros de migración citados anteriormente, el coeficiente de difusión (en estos capítulos se calculó el coeficiente de difusión efectivo (D_E), que es el D en el sistema polímero/alimento) y el $K_{P/F}$, de varios migrantes en sistemas compuestos por un film de polietileno de baja densidad (LDPE) y diferentes alimentos. Estos datos son de gran interés, pues estos parámetros obtenidos a partir de alimentos reales son escasos en la bibliografía.

Los alimentos elegidos fueron siete en total y se seleccionaron de manera que cada uno fuera representativo de un grupo más amplio de alimentos. Los alimentos seleccionados fueron: **queso Gouda** (alimentos sólidos con elevado contenido graso y proteico, y un contenido significativo de agua); **jamón york** (alimentos sólidos con elevado contenido proteico y de agua, con un contenido intermedio de grasa); **fiambre de pavo** (alimentos sólidos con elevado contenido proteico y de agua, con bajo contenido en grasa); **zumo de naranja con pulpa** (alimentos líquidos acuosos de carácter ácido, con sólidos en suspensión, elevado contenido de hidratos de carbono y contenido en grasa muy reducido); **vino tinto** (alimentos líquidos alcohólicos, sin contenido en grasa); **pate** (alimentos semisólidos con elevada viscosidad y con un gran contenido proteico, de grasa y de agua); **salsa de tomate** (alimentos semisólidos con elevado contenido en agua y contenido muy bajo en grasa y proteínas); **crema de**

chocolate (alimentos semisólidos con elevada viscosidad y con un elevado contenido de grasa pero contenido muy bajo de agua).

Siguiendo el mismo criterio, fueron elegidos distintas sustancias modelos que son potenciales migrantes. Las sustancias elegidas fueron: benzofenona (BZP); 1,4-difenilbutadieno (DPBD); Uvitex® OB; difenil ftalato (DPP); isopropiltioxantona (ITX); butilhidroxitolueno (BHT); y triclosan. Son un grupo de sustancias con distintas propiedades entre las cuales destacan por su influencia en el proceso de migración el peso molecular ($182 < MW < 430$ $g \cdot mol^{-1}$) y el coeficiente de partición octanol/agua ($3.18 < \log P\ (o/w) < 7.22$). Además de estas diferencias, las sustancias estudiadas también difieren en grupos funcionales, estructura y geometría molecular, punto de ebullición y fusión, presión vapor, así como en muchas otras características.

En relación al material plástico, solo se utilizó un tipo, el LDPE. Esta poliolefina es un material ampliamente usado en la industria de envasado alimentario y, como las poliolefinas en general, es un material que, comparado con otros polímeros, normalmente favorece el fenómeno de migración. Por eso, al usar este material, se usan condiciones que contribuyen para un escenario de peor caso posible. Para cuestiones de seguridad, esto es ventajoso, ya que nos da un margen de seguridad.

Los migrantes fueron divididos en grupos y añadidos al LDPE de las siguientes formas:

1) En el caso del BZP, DPBD y Uvitex® OB, la incorporación en el polímero se hizo por inmersión de éste en una solución concentrada de las tres sustancias (5000 $mg \cdot L^{-1}$ de BZP y 2000 $mg \cdot L^{-1}$ de DPBD y Uvitex® OB) en etanol (EtOH) durante 48 h a 70 ºC;
2) El LDPE con DPP, ITX y BHT, ya fue adquirido con las sustancias añadidas en su matriz, habiendo realizado la incorporación por extrusión;
3) El caso del triclosan, esta sustancia también fue añadida durante el proceso de extrusión del polímero.

En el capítulo 2 de esta tesis doctoral se describe un método para estudiar la cinética de migración de tres sustancias (BZP, DPBD y Uvitex® OB) entre un film de LDPE y tres alimentos (queso Gouda, zumo de naranja y fiambre de pavo), en el cual se lleva a cabo la cuantificación de las sustancias modelo a través de un método de cromatografía líquida de alta resolución (HPLC) con detección ultravioleta (UV). Al contrario de lo habitual en el estudio de cinéticas de migración, donde las sustancias modelo son cuantificadas a partir del alimento o simulante utilizados, la cuantificación se hizo a partir del polímero, después de haber estado en contacto con uno de los alimentos seleccionados en condiciones de tiempo y temperatura controlados. La cuantificación de los migrantes desde el polímero es una alternativa interesante, ya que es mucho más rápida y sencilla que extraer y cuantificar a partir de los alimentos.

La cinética se hizo por contacto directo, por los dos lados, del film de LDPE con los alimentos. Dependiendo del tipo de alimento, el film fue colocado entre dos lonchas de alimento (en el caso de los alimentos sólidos) o por inmersión (en el caso de los alimentos líquidos). Los sistemas LDPE/alimento se almacenaron en condiciones de temperatura y tiempo controlados. Las temperaturas testadas fueron 20, 40 y 60 ºC. El migrante fue extraído con EtOH a partir del LDPE y posteriormente cuantificado por HPLC acoplado con un detector de díodos en línea (DAD). La separación cromatográfica se hizo con una columna Kromasil C18 (250 × 3.20 mm, 5 µm) termostatizada a 30 ºC y la elución se llevó a cabo en gradiente con dos solventes distintos: agua ultrapura y una solución de THF 30% en metanol (MeOH) (v/v). La detección se hizo a la longitud de onda de 256, 330 y 372 nm para la BZP, DPBD y Uvitex® OB, respectivamente.

Conociendo los concentraciones del migrante en el alimento (calculado como la diferencia entre la cantidad inicial y cantidad final en el polímero), se ajustaron los datos experimentales al modelo matemático propuesto y se determinaron los D_E y los $K_{P/F}$. Aplicando una ecuación de tipo Arrhenius, se testó

la linealidad del D_E en función de la temperatura. Los D_E experimentales fueron también comparados con D_P^* calculados por una ecuación de Piringer.

Los parámetros de migración calculados muestran una gran dependencia tanto del tipo de alimento como del tipo de migrante. De manera general, la migración es más rápida (mayor D_E) y más extensa (menor $K_{P/F}$) con la disminución de la masa molecular del migrante y el aumento del contenido en grasa en el alimento.

Se comprobó la linealidad del coeficiente de difusión con la temperatura, lo que permite determinar este parámetro para cualquier temperatura dentro del rango experimental. No se encontró relación entre temperatura y $K_{P/F}$. Comparando D_E experimental con D_P^*, D_E siempre ha sido inferior. Sin embargo, se demuestra que, cuanto más baja la temperatura, el valor de D_P^* se acerca al de D_E hasta que, eventualmente, D_P^* será inferior al D_E y dejará de representar un valor sobreestimado.

En el capítulo 3 se estudió la cinética de una única sustancia, entre LDPE y un total de siete alimentos: queso Gouda; jamón york; fiambre de pavo; zumo de naranja con pulpa; vino tinto; pate; y salsa de tomate.

La cinética de migración y extracción del LDPE se hizo de la misma forma que en el capítulo anterior. En el caso del paté, un alimento semisólido, el film de LDPE se puso en contacto por inmersión, pero la cantidad de alimento usado fue inferior a la usada con los alimentos líquidos.

La cuantificación se hizo por HPLC-DAD con una columna Kromasil C18 (250 × 3.20 mm, 5 µm) y con una elución en gradiente con agua ultra pura y acetonitrilo (ACN). La detección se hizo a 256 nm.

Se calcularon los parámetros de migración D_E y $K_{P/F}$, y se encontró una buena correlación entre los parámetros experimentales y el modelo sugerido.

Se demostró que el contenido de grasa del alimento es un factor que afecta enormemente la transferencia de sustancias desde el envase al alimento. Los valores que indican una mayor migración (mayor D_E y menor $K_{P/F}$) se obtuvieron en los alimentos con mayor cantidad de grasa, sin embargo en los alimentos con poca o sin grasa en su composición sucedió lo contrario.

En el capítulo 4, a diferencia del anterior, donde se estudió el efecto del tipo de alimento usado en el ensayo de migración, se enfoca sobre todo en las sustancias modelo utilizadas y como sus propiedades intervienen en el fenómeno de migración. Por eso, en este estudio, se utilizaron dos alimentos (paté y crema de chocolate) y un total de seis migrantes modelo (BZP, DPBD, Uvitex® OB, DPP, ITX y BHT).

El procedimiento para la preparación de la cinética de migración y extracción fue idéntico al de los capítulos 2 y 3. La cuantificación de los migrantes BZP, DPBD y Uvitex® OB se realizó de la misma manera que la usada en el capítulo 2, ya que se tratan de los mismos migrantes. Para las restantes sustancias se utilizó una fase móvil compuesta por mezclas de agua ultrapura y ACN. La detección del DPP y BHT se hizo por DAD a una longitud de onda de 205 nm para ambos y, en el caso del ITX, la detección se hizo por fluorescencia con un longitud de onda de excitación de 250 nm y de emisión de 410 nm.

En estos casos, la migración ha sido muy rápida y la mayor parte de las sustancias han migrado en cantidades importantes hacia los alimentos. Esto era lo esperado, dado que se trata de alimentos con una gran cantidad de grasa. Sin embargo, en la crema de chocolate, los $K_{P/F}$ son más bajos. La causa aparente

para esto es el hecho que este alimento tiene un contenido en agua muy bajo y, por tanto, una elevada proporción grasa/agua. Esto indica, que aunque la grasa sea un factor determinante que favorece la migración, la relación grasa/agua también es importante.

En relación a las propiedades de las sustancias, como era de esperar, la que migra en mayor cantidad y más rápido es la BZP que tiene, de entre todas las sustancias seleccionadas, el menor peso molecular y menor log P (o/w). Por otro lado, la que migra menos y más lentamente es el Uvitex® OB que es la que tiene mayor peso molecular y mayor log P (o/w). Esto indica que estos son dos factores fundamentales en la migración hacia alimentos grasos.

En los capítulos 5 y 6 se determinan los parámetros esenciales para estimar la migración. En el capítulo 5 las sustancias modelo utilizadas fueron la BZP, DPBD y Uvitex® OB, y los alimentos fueron el vino tinto, salsa de tomate y jamón york; mientras en el capítulo 6 se utilizaron los migrantes ITX, DPP y BHT, y los alimentos queso Gouda, jamón york y jamón de pavo.

La parte experimental se hizo como en los capítulos anteriores. Después de poner el LDPE aditivado en contacto directo con los alimentos (estilo sándwich en caso que fuera alimento sólido o por inmersión en el caso que fuera un líquido) en condiciones de tiempo y temperatura controlados, los migrantes fueron extraídos del polímero y cuantificados por HPLC-DAD-FLD. Los datos experimentales se ajustaron bien al modelo matemático sugerido y se calcularon los parámetros D_E y $K_{P/F}$.

Los migrantes que tienen tendencia lipofílica presentan un $K_{P/F}$ más bajo en los alimentos con mayor cantidad de grasa en su composición. Para este tipo de alimentos, el D_E es claramente superior, sugiriendo una vez más que el contenido

en grasa de los alimentos es un factor determinante para la migración. De la misma forma, una baja masa molecular también favorece la migración.

Se comprobó la linealidad del D_E en función de la temperatura y se comparó el D_E experimental con el D_P^* calculado teóricamente a través de la ecuación de Piringer. Para las temperaturas usadas en los ensayos, D_E siempre fue inferior que D_P^*, pero, al comprobar la linealidad de los coeficientes de difusión y al determinar la energía de activación y el factor pre-exponencial se ve que, en algunos casos, el D_E superará D_P^* a temperaturas normales de uso. Esto pasa, por ejemplo, con la BZP en el jamón york, donde D_E supera el D_P^* a los 12.8 ºC (asumiendo que la linealidad se mantiene debajo de los 20 ºC).

Los capítulos 7 y 8 enfocan el estudio de la liberación de bisphenol A (BPA) desde biberones de policarbonato (PC) o, más concretamente, el efecto de los detergentes y de varias aminas en la liberación de esta sustancia. El capítulo 7 se centra en el efecto de los detergentes, mientras el 8 se centra en el efecto de las aminas. También se ensayó la lejía y soluciones de hidróxido de sodio con distintos valores de pH.

El PC era un polímero muy usado en la producción de biberones y es compuesto por unidades monoméricas de BPA. Muchos estudios científicos ya informaron acerca de la liberación de BPA a partir del PC, debiéndose sobre todo a la degradación del PC y no a la migración del monómero. Esta información es bastante alarmante debido a la existencia de estudios que sugieren que el BPA puede tener diversos efectos en la salud (actividad estrogénica, desordenes al nivel del metabolismo, problemas neurológicos y comportamentales, cancerígeno, etc.), siendo especialmente grave en los bebés que son más susceptibles a estos efectos debido a su dieta y a que su metabolismo no está aun completamente desarrollado.

Se tomaron muestras de biberones, que se colocaron en soluciones de distintos detergentes y distintas soluciones acuosas de aminas, y se incubaron en condiciones de tiempo y temperatura controlados. Al largo del tiempo se tomaron dos muestras para comprobar si estas sustancias tienen o no algún efecto sobre la liberación de BPA, la primera después de 1 hora a 120 ºC y la segunda después de 120 horas a 25 ºC. Después de esto, para comprobar si la liberación del monómero seguía una vez eliminado el detergente o las aminas, se lavaron las muestras de biberón con agua destilada y si incubó nuevamente 1 hora a 120 ºC, esta vez solo con agua destilada. Esto se repitió dos veces más.

La cuantificación e identificación del BPA se hizo por HPLC-DAD-FLD y por GC-MS, respectivamente. Para identificar el BPA se usó un método de GC-MS (cromatografía gaseosa con un detector de masas acoplado) y la inyección se hizo en modo *split*. La temperatura del inyector fue 225 ºC, mientras la temperatura de la columna era de 250 ºC (método isocrático). La columna usada fue una DB-5MS de 30 m de largo y 0.25 mm de diámetro interno. La identificación se hizo por comparación del espectro de masas de las muestras con los espectros de masas de soluciones estándar de BPA y, también con los espectros de la librería XCalibur. La cuantificación se hizo por HPLC-FLD usando una columna Kromasil C18 (250 × 3.20 mm, 5 µm) y una fase móvil compuesta por agua ultrapura y ACN.

Por comparación con los resultados obtenidos con una muestra de biberón en contacto con agua y sujeta a las mismas condiciones, se comprobó que la presencia de un detergente, en algunos casos, incrementa la liberación de BPA. En un caso particular, hubo un incremento enorme, superior a 500 veces, y se comprobó que la liberación seguía siendo elevada después de eliminar el detergente.

En el caso de las aminas se observó lo mismo que con los detergentes: en algunas muestras no se observó incremento de la liberación de BPA, mientras en otros casos sí, sobre todo en los casos de aminas lineales de bajo peso molecular. Con algunas aminas, la liberación del monómero llegó a ser miles de veces superior. Los resultados fueron comparados con los obtenidos con soluciones de hidróxido de sodio a distintas concentraciones para comprobar si el efecto apenas se debía al incremento del pH, descartándose tal relación.

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1 Introducción general

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1.1 Envases alimentarios

El Hombre siempre ha sentido la necesidad de conservar y proteger los alimentos que tiene en exceso como una medida preventiva para épocas de escasez o de mayor necesidad. Los animales, los insectos, la imprevisibilidad de la cosecha y de la caza, el deterioro de los alimentos (por microorganismos, cambios físicos y químicos) son algunos problemas que podían amenazar la supervivencia de nuestros antepasados.

En la actualidad, una correcta preservación de los alimentos sigue siendo necesaria y es un asunto de extrema importancia para que tengamos acceso a alimentos nutritivos y saludables. En nuestra sociedad, la mayoría de la población no se encuentra cerca de los lugares de producción de los alimentos, siendo también necesario transportarlos largas distancias y tenerlos disponibles en gran cantidad durante todo el año, independientemente de la época en que se produzcan. Para cumplir estos objetivos, se usan varias técnicas siendo el uso de envases alimentarios una de las más empleadas. Sin el uso de envases alimentarios el mercado de consumo tal y como lo conocemos sería virtualmente imposible.

Para gran parte de los consumidores, el envase de los alimentos (o envases en general) consiste en un simple recipiente de estos productos, a veces visto como innecesario o excesivamente complejo (Lee, Yam y Piergiovanni, 2008). Sin embargo es más que un mero contenedor. Los envases alimentarios son el resultado de un largo proceso de búsqueda y desarrollo continuado destinado a

hallar mejores métodos y técnicas de envasado. Según el Instituto de Británico de Envasado (UK Institute of Packaging), envase se define como:

1. un sistema coordinado de preparación de mercancías para su transporte, distribución, almacenamiento, comercialización y uso final;
2. un medio para asegurar la entrega segura al consumidor final en condiciones sanas a un costo mínimo;
3. una función técnico-económica con vistas a minimizar los costos de la entrega mientras maximiza las ventas (y, por lo tanto, los beneficios).

Otra definición de envases, según el Instituto Internacional del Embalaje (Packaging Institute International), es “la envoltura de productos, artículos o paquetes en una bolsa, caja, taza, bandeja, lata, tubo, botella u otra forma de recipiente para llevar a cabo una o más de las siguientes funciones: contención, protección, conservación, comunicación, utilidad y funcionalidad; Si el dispositivo o contenedor realiza una o más de estas funciones, se considera un envase” (Bramklev, Olsson, Orremo y Wallin, 2001).

Se trata de un sistema complejo y multidisciplinar que implica muchas áreas técnicas (marketing, diseño, desarrollo de envases, uso de maquinaria específica para la producción, etc.), muchos tipos de embalajes (latas de metal, frascos de vidrio, bolsas de plásticos o de papel, cajas de cartón y muchos otros) y que se destina a una entrega eficiente de alimentos seguros y de buena calidad a través de toda la cadena de suministro, hasta el momento del consumo del alimento y eliminación/reciclaje del envase. Como tal, los objetivos de los envases son muy amplios y dependen mucho del alimento o producto al que se destinan.

Al elegir un envase alimentario hay que tener en cuenta varios puntos, siendo el principal, la protección eficaz del alimento envasado durante su vida útil.

1.1.1 Funciones de los envases alimentarios

Independientemente del alimento, un envase efectivo es fundamental. Para que sean efectivos, los embalajes necesitan cumplir una o más de las siguientes funciones (Robertson, 1993):

- *Contención*: probablemente la función más obvia y principal del envase; una correcta contención del alimento va a depender de sus características (por ejemplo, si es un sólido o un líquido, las dimensiones de las partículas del alimento); va aislar el producto del medio ambiente, facilitando su transporte y evitando contaminación de la comida o polución del ambiente;
- *Protección y preservación*: proteger el alimento de daños físicos, adulteración, deterioro fisicoquímico (humedad, calor, luz), microbiológico (bacterias, moho) u otros factores externos (si las causas de deterioro son internas, un envase clásico no puede proteger el alimento) desde la producción hasta el consumo, evitando perdidas de propiedades organolépticas y de nutrientes, haciendo que el alimento sea seguro para el consumidor y alargando la vida útil del alimento; esta es la característica más importante del embalaje y va depender de la estabilidad y fragilidad del producto, del tiempo de vida útil pretendido y de las condiciones de hermeticidad necesarias.
- *Conveniencia*: para agradar a los consumidores y, al mismo tiempo, contribuir para el éxito comercial del producto, los embalajes necesitan ser prácticos, sin que eso incremente el coste o disminuya la calidad del producto alimenticio; algunos ejemplos de conveniencia son apertura fácil, ergonomía de los envases, posibilidad de usar en el microondas, envases

auto-enfriable y auto-calentable, envases con dosis individuales; facilidad de transporte y almacenamiento;

- *Comunicación*: los envases, además de identificar el alimento e informar cuales son sus ingredientes (requisito legal) o de proporcionar instrucciones de cómo manipularlo y prepararlo, también promueven la marca e influencian la decisión de los consumidores en el momento de la compra; en el mercado global en que vivimos actualmente, donde un producto puede ser vendido en países de idiomas distintos, la identificación de los productos por colores, símbolos e imágenes es muy importante; por último, el uso del sistema de código de barras permite identificar los productos de una manera rápida y eficaz, siendo una herramienta muy útil en las tiendas, superficies comerciales, almacenes y distribuidores.

Estas funciones necesitan ser aplicadas en tres tipos de entornos distintos, sino el envase no será eficaz en términos de protección, de costes o de satisfacción del consumidor. Estos entornos son (Wagner y Sugden, 2009):

- *Físico*: donde el alimento es susceptible de sufrir daños físicos (por golpes, vibración, perforación, compresión) durante el período de almacenamiento, transporte y uso en casa
- *Ambiental*: ambiente que rodea el alimento y que le puede causar daño como resultado del contacto con gases, humedad, suciedad, luz, variaciones de temperatura, microrganismos, entre muchas otras potenciales causas de daños
- *Humano*: es la interacción entre el envase y nosotros, y se centra en dos aspectos referidos anteriormente, la información y conveniencia.

Un buen conocimiento de las funciones del envase y de los distintos ambientes que lo afectan es esencial para desarrollar embalajes apropiados y eficaces, trayendo ventajas a nivel económico, nutricional y de salud pública (Robertson, 1993; Lee, Yam y Piergiovanni, 2008).

Aparte de las funciones citadas anteriormente, la industria alimentaria busca nuevas formas e ideas para desempeñar mejor esas funciones. El área donde se encontraron más soluciones para esta tarea fue en los materiales y objetos activos e inteligentes en contacto con alimentos. Estos dos conceptos se definen de la siguiente forma (Reglamento (CE) no 1935/2004):

- materiales y objetos activos en contacto con alimentos: “los materiales y objetos destinados a ampliar el tiempo de conservación, o a mantener o mejorar el estado de los alimentos envasados, y que están diseñados para incorporar deliberadamente componentes que transmitan sustancias a los alimentos envasados o al entorno de éstos o que absorban sustancias de los alimentos envasados o del entorno de éstos“
- materiales y objetos inteligentes en contacto con alimentos: “los materiales y objetos que controlan el estado de los alimentos envasados o el entorno de éstos”

1.1.2 Envases de plástico

Los envases pueden ser de varios materiales, entre los cuales encontramos papel, cartón, madera, metal, vidrio, cerámica, plástico. Cada uno de estos materiales presenta propiedades diferentes, que deben ser tenidas en cuenta en

la elección del material de envasado en función de varios factores. De estos materiales, el más utilizado es el papel y cartón, pero aquellos cuyo uso se ha incrementado más en las últimas décadas han sido los plásticos (EUROPEN, 2013).

Los plásticos, según el Reglamento (UE) nº 10/2011 de la Comisión de 14 de enero de 2011, se definen como "polímero al que pueden haberse añadido aditivos u otras sustancias y que es capaz de funcionar como principal componente estructural de materiales y objetos finales", mientras polímero se define como "toda sustancia macromolecular obtenida por: (a) un procedimiento de polimerización, como poliadición o policondensación, o cualquier otro procedimiento similar, a partir de monómeros y otras sustancias de partida; (b) modificación química de macromoléculas naturales o sintéticas; o (c) fermentación microbiana".

Los plásticos poseen un conjunto de características que hace que sean un material muy atractivo para la producción de envases: son extraordinariamente versátiles y fácilmente adaptables para satisfacer necesidades técnicas específicas; moldeable, posibilitando una gran disponibilidad de tamaños y formas; opciones de transparencia o color; baja densidad, facilitando el transporte y economizando el consumo de combustible; durabilidad; flexibilidad; higiene; resistencia física y química; aislamiento térmico y eléctrico; buena seguridad; bajo coste de producción; impermeabilidad al oxígeno y vapor de agua; receptividad a la impresión y al recubrimiento metálico; etc. Estas características son la razón del éxito de los polímeros como material de envase alimentario (Jenkins y Harrington, 1991a).

Los plásticos se pueden clasificar de muchas maneras distintas. De acuerdo con su comportamiento frente al calor, se dividen en dos grupos:

- *Termoplásticos*: material polimérico con cadenas largas conectadas por fuerzas de Van der Waals; al incrementar la temperatura, estas conexiones se rompen y el plástico pasa a un estado de elevada viscosidad, siendo fácilmente moldeable; al enfriar, las conexiones son restablecidas y el polímero se solidifica; así, estos plásticos pueden ser calentados, moldeados con una nueva forma y enfriados varias veces (útil para reciclar); la mayoría de los polímeros empleados en envases alimentarios son de esta clase;

  Ejemplos: Polietileno (PE); poliestireno (PS); poliamida (PA); polietileno tereftalato (PET); policarbonato (PC).

- *Termoestable*: plásticos que han sido formados después de un proceso de reticulación, donde las cadenas poliméricas establecen enlaces covalentes entre sí y, como consecuencia, estos plásticos no se pueden ablandar por calentamiento porque, cuando los enlaces covalentes se rompen por efecto del calor, no se vuelven a formar cuando la temperatura baja (Crosby, 1981; Chanda, 2006).

  Ejemplos: resinas epoxi; siliconas; resinas poliéster; caucho natural vulcanizado o sintético.

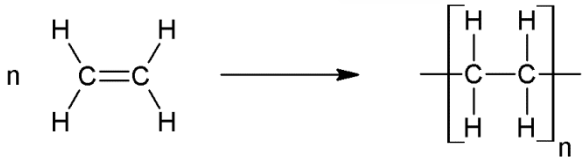
Los plásticos también se pueden clasificar de acuerdo con:

- *Origen*: Natural, sintético y semisintéticos;
- *Composición química*: homopolímero y heteropolímero;
- *Estructura de las cadenas*: lineal, ramificado; reticulado
- Reacción de síntesis: adición, condensación, formados por reacción en cadena, formados por reacción en etapas;
- Estructura molecular: amorfos, semicristalinos, cristalinos;
- Fuerza tensil: plástico, elastómero y fibra.

1.1.3 Ejemplos de polímeros usados en envases alimentarios

Existe un elevado número de distintos polímeros con distintas propiedades y, consecuentemente, con distintas aplicaciones. Se hablará de una forma general de algunos de los más usados en la industria de envase de alimentos.

Polietileno (PE)



Polietileno es una poliolefina (PO; polímero obtenido mediante la polimerización de alquenos) que fue producida por primera vez en 1933 en la Imperial Chemical Industries según un método de polimerización por radicales, un tipo de polimerización por adición, del monómero etileno, un abundante producto secundario de la refinación del petróleo y de otros procesos, a presiones elevadas

(142 MPa) y temperaturas altas (170 ºC). Como resultado, se obtuvo polietileno (PE) (Caraher Jr., 2007).

LDPE es un tipo de PE que, a pesar de su simplicidad, es muy versátil y uno de los plásticos más ampliamente usados, siendo uno de los polímeros más utilizados en la industria de envasado alimentario. Por un bajo coste, puede ser fácilmente moldeado por inyección o por extrusión. Otras ventajas de este polímero son la dureza, flexibilidad, fácilmente utilizado como revestimiento de otros materiales (papel, cartón o aluminio), barrera eficaz al agua y al vapor de agua, reciclable () y buena resistencia térmica, química y al impacto. Este polímero se caracteriza por una densidad de 0.910-0.940 $g \cdot cm^{-3}$. Esta baja densidad relativa es una consecuencia de las ramificaciones de la cadena polimérica, que van a impedir un mayor empaquetamiento del polímero. Además de afectar la densidad, las ramificaciones también afectan la cristalinidad y temperatura de reblandecimiento (Peacock, 2000).

Además de LDPE, existen otros tipos de PE, como el polietileno de alta densidad (HDPE) y polietileno linear de baja densidad (LLDPE).

HDPE es un tipo de PE obtenido a presiones y temperaturas relativamente bajas por un proceso catalítico. Posee una mayor densidad y mayor grado de cristalinidad que el LDPE debido al menor número y menor extensión de sus ramificaciones. Algunas de las ventajas del HDPE frente al LDPE es la resistencia física (dureza, rigidez, impacto), resistencia química, propiedades barrera y punto de fusión más elevado. Sin embargo, puede ser convertido tan fácilmente como el LDPE en film, hoja o envase, y por lo tanto es un material muy versátil.

LLDPE es un copolímero que tiene una densidad similar a la del LDPE, pero una fuerza y resistencia similar al del HDPE. Es producido a través de un método

similar al del HDPE, al cual se añadieron alquenos (por ejemplo 1-buteno, 1-hexeno o 1-octeno). El resultado es una cadena polimérica con un gran número de ramificaciones (como en el LDPE), pero estas ramificaciones son de pequeña extensión (como en el HDPE) (Jenkins y Harrington, 1991b).

Tabla 1.1. Propiedades de los tipos de PE referidos

Nombre		LDPE	HDPE	LLDPE
Estructura		Ramificada con muchas ramificaciones largas	Linear con pocas ramificaciones cortas	Linear con muchas ramificaciones cortas
Densidad (g/cm^3)		0.910–0.940	0.94-0.97	0.900–0.940
Cristalinidad (%)	42-62	62-82	22-62	
Punto de fusión (ºC)	98-115	125-132	100-125	
Punto de ablandamiento (ºC a 455 kPa)	40-44	80-90	55-80	

Peacock (2000)

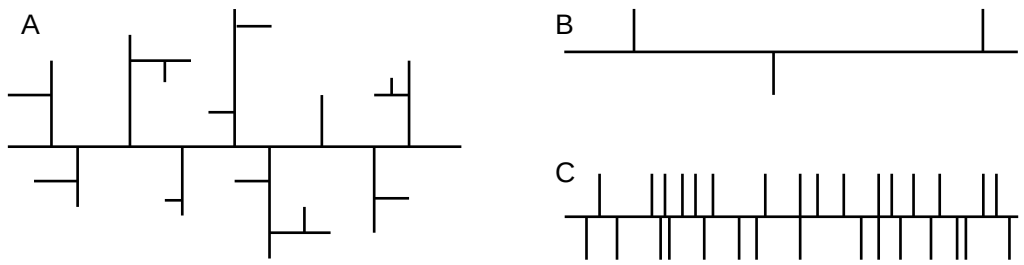
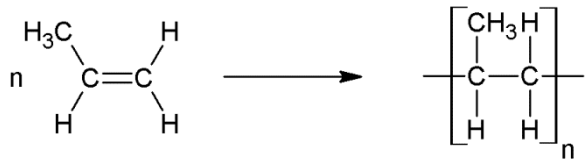


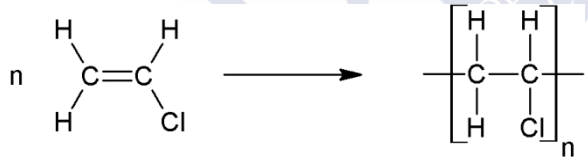
Figura 1.1. *Esquema de la estructura de LDPE (A), HDPE (B) y LLDPE (C)*

Polipropileno (PP)



Tal como el PE, el PP es también una PO, hecha por unidades repetidas de propileno. No es tan robusto como el PE, pero tiene algunas ventajas relativas a él, siendo por lo tanto preferible en algunas situaciones específicas. Tiene una baja densidad (0.90-0.91 $g \cdot cm^{-3}$, una de las densidades más bajas entre los polímeros de uso alimentario), excelente resistencia química, un punto de fusión relativamente elevado (> 160 ºC), una buena resistencia la fatiga y un costo moderado (Brandsch y Piringer, 2000).

Policloruro de vinilo (PVC)

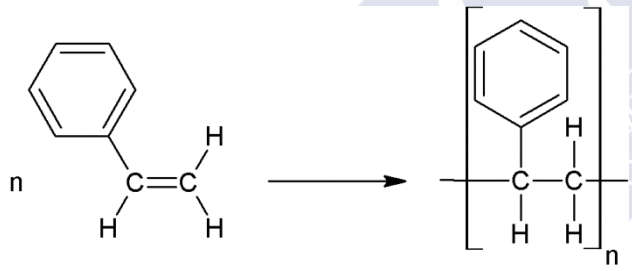


El PVC, resultante de la polimerización del monómero de cloruro de vinilo, está entre los polímeros más complejos y difíciles de producir, ya que requiere de un número importante de ingredientes y un balance adecuado de éstos para poder transformarlo al producto final deseado.

Paradójicamente, el PVC es un polímero de gran éxito y esto se debe a su versatilidad, aunque necesite de varios aditivos en su formulación. A pesar de ser un termoplástico, a partir de él se pueden obtener tanto productos rígidos como productos flexibles.

PVC, al contrario de otros polímeros como el PP o LDPE, es un material naturalmente frágil. Su éxito comercial inicial, en 1930, resultó de la adición de substancias llamadas plastificantes que le proporcionan flexibilidad. Este tipo de PVC es ampliamente usado en cables, juguetes, recubrimientos, film plástico adherente, etc. Más tarde, con el desarrollo de nuevos aditivos, se produjo PVC rígido que actualmente es usado en tuberías (como sustituto de cobre y hierro) y otros materiales de construcción, componentes de coches y como material de envase (Brandsch y Piringer, 2000).

Poliestireno (PS)



Polímero transparente, duro y que no transmite sabores u olores, características que lo convierten en una buena opción como material en contacto con alimentos.

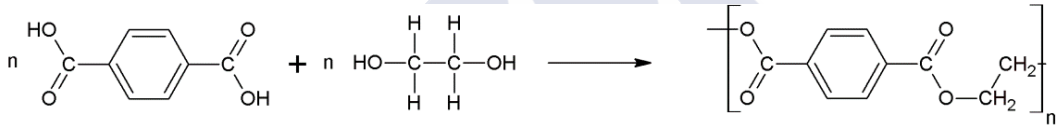
PS es un polímero muy utilizado y relativamente barato que se puede presentar en varias formas y, según el tipo, puede tener distintas aplicaciones:

- PS de uso general (General Purpose Polystyrene - GPPS): duro, claro y generalmente incoloro; muy utilizado en envases alimentarios y utensilios de cocina
- PS de alto impacto (High Impact Polystyrene - HIPS): copolímero que resulta de la adición de caucho o de butadieno, incrementando su

resistencia al impacto y durabilidad; también muy utilizados como material en contacto con alimentos;

- PS expandido (Expanded Polystyrene – EPS) y PS extruido (Extruded Polystyrene – XPS): formas similares del PS que se diferencian por el proceso de conformación caracterizados por la presencia de gas en su constitución y por la consecuente muy baja densidad; generalmente utilizados como aislantes térmicos y acondicionador de productos frágiles (Wagner y Sugden, 2009).

Polietileno tereftalato (PET)



El polímero PET es un poliéster resultado de la reacción de etileno glicol con el ácido teraftálico. Sus propiedades lo convierten en un excelente material de envase de alimentos, debido a su resistencia química, ligereza, estabilidad en un gran rango de temperaturas (soporta temperaturas negativas y temperaturas de cocción, permitiendo que el alimento sea cocinado en el proprio envase), reciclable, elasticidad, seguridad y no transmite olores ni sabores a los alimentos envasados.

El uso del PET se incrementó mucho a partir de la década de 70, cuando empezó a ser empleado en la producción de botellas. Desde entonces, el PET ha sido ampliamente usado como material de botellas de agua, zumos, bebidas carbonatadas y aceites, siendo también usado como recipiente de detergentes, productos farmacéuticos, cosméticos y otros bienes de consumo (Robertson, 1992).

1.2 Interacciones envase-alimento

Como ya ha sido comentado, una de las funciones del envase es impedir que el alimento interactúe con el ambiente, para preservar la calidad y seguridad del producto. Las técnicas de envasado solucionan muchos de estos problemas, pero plantean otros. Sin embargo, los materiales de los envases no son inertes y, consecuentemente, pueden interactuar con el alimento. Algunas de las posibles consecuencias de estas interacciones son: contaminación, toxicidad y pérdida de calidad (nutritiva y organoléptica) del alimento, menor aceptación comercial del producto, menor tiempo de validez del alimento y alteración de las características del material de envase (Cruz, Sanches Silva, Sendón García, Franz y Paseiro Losada, 2007).

Hay tres tipos de interacciones envase-alimento:

- *Migración*: transferencia de masa desde el material de envase al alimento a través de un proceso submicroscópico, siendo este movimiento controlado por difusión molecular (movimiento browniano) (Katan, 1996)
- *Sorción*: transferencia de sustancias desde el alimento al envase, causando pérdidas de aromas y de sabor.
- *Permeación*: intercambio de sustancias como gases y vapores entre el ambiente y el alimento a través del envase.

Este trabajo se va a centrar en el primero, la migración. De los tres fenómenos mencionados, la migración es aquel que plantea más preocupaciones desde el punto de vista de seguridad alimentaria, debido al potencial efecto nocivo que algunas sustancias pueden ocasionar en la salud del consumidor y, por eso, es la interacción envase-alimento más estudiada.

La exposición del consumidor a sustancias químicas no deseables en los alimentos como resultado de la migración desde el envase es un aspecto de extrema importancia para la salud pública y que ha despertado el interés de la Unión Europea (UE). En este contexto, fue creado el proyecto Europeo FACET (Flavourings, Additives and food Contact materials Exposure Task), que tiene como objetivo "la creación de un sistema de vigilancia de exposición de sustancias química de los alimentos (…) que abarca las regiones representativas de la UE y que reunirá, al más alto nivel posible, las necesidades de las autoridades reguladoras de la UE en materia de protección de la salud de los consumidores" (FACETa).

1.3 Migración en materiales plásticos

Los plásticos, que están constituidos por polímeros (substancias compuestas por moléculas de elevada masa molecular relativa, cuya estructura comprende esencialmente la repetición múltiple de unidades derivadas, real o conceptualmente, de moléculas de baja masa molecular relativa) (IUPAC), poseen varias sustancias que no están covalentemente conectadas a la matriz polimérica (ejemplos: aditivos, monómeros residuales, oligómeros, sub-productos de reacción, productos de reacciones secundarias, impurezas de la materia prima, coadyuvantes de polimerización, productos resultantes de la degradación del polímero y otros) y que, por eso, son capaces de desplazarse desde el interior del polímero hasta el alimento y, por medio de la ingesta de los mismos, entran en el organismo donde pueden ocasionar efectos perjudiciales a la salud.

La migración es un fenómeno que depende de varios factores:

- Polímero: tipo de polímero, densidad, cristalinidad, punto de ablandamiento
- *Alimento (o simulante)*: estado físico, composición (porcentajes de grasa, agua, etc.), y la proporción de estos
- *Sustancia*: peso molecular, solubilidades de la sustancia en el alimento y polímero, polaridad
- Temperatura y tiempo del contacto entre el plástico y el alimento

1.4 Modelos matemáticos usados en la estimación de la migración

La migración es un proceso físico extremamente complejo que, como ya fue referido, depende de varios factores. Sin embargo, este es un fenómeno predecible que puede ser descrito por la Segunda Ley de Difusión de Fick (Ec. 1.1):

$$\frac{\partial C_P}{\partial t} = D_P \frac{\partial^2 C_P}{\partial x^2} \qquad (\textit{Ecuación 1.1})$$

Donde: $\mathbf{C_P}$ ($mg \cdot kg^{-1}$) es la concentración de la sustancia en el polímero al tiempo **t** (s) y distancia **x** (cm) que es la distancia recorrida por la sustancia a partir de la interfase polímero-alimento (o simulante); $\mathbf{D_P}$ ($cm^2 \cdot s^{-1}$) es el coeficiente de difusión en el polímero.

Varias soluciones analíticas y numéricas de la Ec. 1.1 fueron sugeridas por diversos autores. Estas soluciones se basan en asunciones que, cuando son expresadas en términos matemáticos, nos permite calcular la concentración del migrante en el alimento en cada momento (Chatwin y Katan, 1989). Estas soluciones pueden ser herramientas útiles para propósitos legislativos y de regulación.

Uno de los autores que hizo un trabajo extensivo en esta área fue J. Crank (1975). Teniendo en cuenta distintos escenarios, Crank sugirió varias soluciones de la Ec. 1.1. Para una difusión unidimensional desde un film de volumen finito y espesor constante a una solución en constante agitación (para que la sustancia esté igualmente distribuida en el alimento o simulante) y de volumen también finito, Crank (1975) propuso una solución que, después de una pequeña modificación, se puede expresar de la seguinte forma (Simoneau, 2010):

$$\frac{M_{F,t}}{A} = c_{P,0}\rho_P d_P \left(\frac{\alpha}{1+\alpha}\right) \times \left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-D_P t \frac{q_n^2}{d^2}\right)\right] \qquad (\textit{Ecuación 1.2})$$

Con:

$$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P} \qquad \text{y} \qquad K_{P/F} = \frac{c_{P,\infty}}{c_{F,\infty}} \frac{\rho_P}{\rho_F}$$

Donde: $\mathbf{M_{F,t}}$ (µg) es la cantidad total del migrante que fue transferido entre el polímero (P) y el alimento (F) al final del tiempo **t** (s); **A** (cm^2) es la área de contacto entre P y F; $\mathbf{c_{P,0}}$, $\mathbf{c_{P,\infty}}$ y $\mathbf{c_{F,\infty}}$ ($\mu g \cdot g^{-1}$) son las concentraciones del migrante en P al tiempo 0, en P cuando se llega al equilibrio en el sistema, y en F en el equilibrio,

respectivamente; $\boldsymbol{\rho_P}$ y $\boldsymbol{\rho_F}$ ($g \cdot cm^{-3}$) son la densidad de P y de F, respectivamente; $\mathbf{d_P}$ (cm) es el espesor de P; $\mathbf{V_P}$ y $\mathbf{V_F}$ (cm^3) son el volumen P y F, respectivamente; $\mathbf{q_n}$ es el número de raíces de la ecuación tan $q_n = -\alpha\, q_n$; $\mathbf{D_P}$ ($cm^2 \cdot s^{-1}$) es el coeficiente de difusión del migrante en P; $\mathbf{K_{P/F}}$ es el coeficiente de partición del migrante entre P y F; $\boldsymbol{\alpha}$ es la relación entre la cantidad de migrante en F y en P, cuando se llega al equilibrio.

Esta solución es válida si se presupone que:

- la sustancia modelo esté homogéneamente distribuida en el polímero;
- no hay resistencia en la transferencia de migrante entre el polímero y el alimento en la interface de estos dos elementos que componen el sistema;
- durante el proceso de migración, las interacciones entre el envase y alimentos son negligibles, y no hay *swelling* de P por captación o absorción de F;
- el $K_{P/F}$ es conocido y está bien definido.

Para aplicar esto modelo, son necesarios dos parámetros esenciales:

- D_P – representa la cinética del proceso, la velocidad a que el migrante se mueve en la matriz polimérica;
- $K_{P/F}$ – expresa el equilibrio termodinámico del proceso de migración, o sea, la proporción entre la concentración del migrante en el polímero y en el alimento cuando se alcanza el estado de equilibrio (Granda-Restrepo *et al*, 2009; Tehrany y Desobry, 2004).

Si el modelo matemático se aplica a un sistema envase-alimento (o simulante), D_P se puede sustituir por D_E (coeficiente de difusión efectivo). Mientras D_P refleja el comportamiento de difusión dentro del polímero, D_E describe la difusión en el sistema, es decir, entre el material en contacto con alimentos (FCM – Food Contact Material) y el alimento o simulante (Sanches Silva, Cruz Freire, Sendón García, Franz y Paseiro Losada, 2007).

La Ec. 1.2 no siempre es la más adecuada. “Cuando son necesarios más de tres o cuatro términos es mejor usar una solución alternativa” (Crank, 1975), que es una simplificación de la ecuación citada anteriormente:

$$\frac{M_{F,t}}{M_{F,\infty}} = (1+\alpha)\left[1 - e^{\left(\frac{\tau}{\alpha^2}\right)} erfc\left(\frac{\tau}{\alpha^2}\right)^{\frac{1}{2}}\right] \qquad (\textit{Ecuación 1.3})$$

Con:

$$\tau = \frac{D_t}{d_P^2}$$

Donde: $\mathbf{M_{F,\infty}}$ (µg) es la cantidad total del migrante que fue transferido desde el P al F cuando el sistema ya ha llegado al estado de equilibrio; **τ** es un parámetro especifico del polímero relacionado con la “energía de activación”

Los parámetros de migración D_E y $K_{P/F}$ determinados en esta tesis fueron calculados ajustando el modelo matemático de la Ecuación 1.3 a los datos experimentales.

Dado que D (coeficiente de diffusión), es dependiente de la temperatura (Sanches Silva, Cruz Freire y Paseiro Losada, 2010), para testar la relación entre D y la temperatura, se usa la siguiente ecuación de Arrhenius:

$$D = D_0 e^{-\frac{E_A}{RT}} \qquad \text{(}\textit{Ecuación 1.4}\text{)}$$

Donde: $\mathbf{D_0}$ ($cm^2 \cdot s^{-1}$) factor pre-exponencional que corresponde al valor teórico de D a temperatura infinita; $\mathbf{E_A}$ ($kJ \cdot mol^{-1}$) es la energía de activación; **R** ($8.31 \times 10\text{-}03\ kJ \cdot mol^{-1} \cdot K^{-1}$) es la constante de los gases ideales; y **T** (K) es la temperatura.

Tomando el logaritmo natural de la Ecuación 1.4:

$$lnD = -\frac{E_A}{R}\frac{1}{T} + lnD_0 \qquad \text{(}\textit{Ecuación 1.5}\text{)}$$

En caso que haya linealidad entre los D a distintas temperaturas, esta ecuación también se puede usar para prever el valor de D.

La determinación experimental de los parámetros de migración es un trabajo que consume gran esfuerzo y tiempo (p. ej. desarrollo de métodos analíticos, preparación de los films, ensayos de migración a lo largo del tiempo, extracción y cuantificación de las sustancias modelo). Para evitar este

inconveniente se desarrollaron varios métodos teóricos y diversos modelos matemáticos que permiten estimar los coeficientes de difusión. Sin embargo, estos modelos son tan sofisticados que hace que su aplicación sería tan o más difícil, y menos exacto, que la propia determinación experimental de la migración exigida por la legislación (Mercea, 2000).

En busca de una ecuación sencilla que nos permita estimar el coeficiente de difusión en distintos polímeros sin que sea necesario cualquier dato experimental, Piringer y colaboradores desarrollaron un modelo sencillo que establece una relación empírica entre la masa molecular relativa del migrante, la temperatura y el tipo de polímero utilizado (Piringer, 1994; Brandsch, Mercea y Piringer, 2000; Begley *et al*, 2005):

$$D_P^* = 10^4 \exp\left(A_P - 0.1351\, M_r^{2/3} + 0.003\, M_r - \frac{10454}{T}\right) \qquad \textit{(Ecuación 1.6)}$$

Con:

$$A_P = A'_P - \frac{\tau}{T}$$

Donde: $\mathbf{D^*_P}$ ($cm^2 \cdot s^{-1}$) límite superior específico del coeficiente de difusión en P; $\mathbf{A_P}$ es un parámetro que describe el comportamiento de difusión de los migrantes en P; $\mathbf{A'_P}$ es un parámetro de difusión relacionado con P e independiente de la temperatura; $\boldsymbol{\tau}$ es un parámetro específico del polímero relacionado con la "energía de activación"; $\mathbf{M_r}$ (Da) es la masa molecular relativa del migrante; **T** (K) es la temperatura. Para el LDPE $\mathbf{A'_P}$ = 11.5 y $\boldsymbol{\tau}$ = 0.

A pesar de este modelo no ser exacto al medir el D_P real, es extremadamente útil debido a su fácil y rápida aplicación, no requiere pruebas y, una vez que calcula valores de D_P sobrestimados, estos pueden ser usados para pruebas de seguridad (Reynier, Dole y Feigenbaum, 1999).

En relación al $K_{P/F}$, este parámetro también se puede calcular según la ecuación:

$$K_{P/F} = \frac{C_{P,\infty}}{(C_{P,0} - C_{P,\infty})} \times \frac{W_F}{W_P} \qquad (\textit{Ecuación 1.7})$$

Donde: $\mathbf{C_{P,0}}$ y $\mathbf{C_{P,\infty}}$ ($\mu g \cdot g^{-1}$) es la concentración del migrante en el polímero al tiempo 0 y en el estado de equilibrio, respectivamente; $\mathbf{W_P}$ y $\mathbf{W_F}$ es la masa del polímero y la masa de alimento (g)

Nota: para calcular el $K_{P/F,}$ la concentración se suele expresar en función del volumen. En la Ec. 1.7 pero, por cuestiones prácticas, esta se expresa en funcion de la masa porque muchas veces es muy complicado determinar el volumen de los alimentos. Si se expresa $K_{P/F}$ en función del volumen es necesario multiplicar la Ec 1.7 por ($\boldsymbol{\rho}_P \boldsymbol{\rho}_F^{-1}$) (g cm^{-3}).

Esta es una forma bastante sencilla de calcular este parámetro sin que sea necesario proceder a la determinación del migrante en el alimento (y, consecuentemente, evitar procedimientos trabajosos y demorados). Sin embargo, para aplicar este procedimiento, es necesario utilizar un punto experimental de la

cinética y, si aún no se llegó al estado de equilibrio o si hay algún desvío entre el valor experimental y el valor real, esta ecuación podría dar un valor de $K_{P/F}$ erróneo.

Para que el modelo de migración pueda ser considerado fiable es necesario que exista una buena correlación entre los valores estimados y los datos experimentales. Para medir la proximidad entre los datos obtenidos experimentalmente y los obtenidos a través de los modelos sugeridos (Ec. 1.2 y 1.3), se calcula la raíz cuadrada del cuadrado medio del error (RMSE – root mean squareed error):

$$RMSE\ (\%) = \frac{1}{M_{P,0}} \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(\left(M_{F,t}\right)_{exp,i} - \left(M_{F,t}\right)_{pred,i} \right)^2} \times 100 \qquad (\textit{Ecuación 1.8})$$

Donde: **n** es el número de puntos experimentales usados en la cinética de migración; **i** es el número de observaciones.

1.5 Legislación

Con la creación de la Comunidad Económica Europea en 1957, se volvió necesario proceder a la armonización de la legislación de los distintos estados miembros. La legislación relacionada con los materiales de contacto con alimentos no fue excepción.

El 15 de enero de 2011, fue publicado el Reglamento (UE) Nº 10/2011 de la Comisión, de 14 de enero de 2011, sobre materiales y objetos plásticos destinados a entrar en contacto con alimentos. Este reglamento es aplicable a partir del 1 de mayo de 2011, pero no será obligatoriamente aplicado en su totalidad hasta el 1 de enero de 2016.. Hasta esta fecha, los diferentes artículos irán entrando en vigor en distintas fases, como está descrito en el capítulo VI del Reglamento (UE) Nº 10/2011.

El reglamento 10/2011 de 14 de enero de 2011 tiene como objetivo actualizar y unificar toda la legislación comunitaria existente relativa a plásticos en contacto con alimentos en un único texto e incluye listas positivas de sustancias de partida; ensayos de migración (específica y global); declaración y realización de ensayos de conformidad; requisitos generales, restricciones y especificaciones aplicables a las sustancias, materiales y objetos plásticos; etc. Los principales cambios relativos a las directivas anteriores son:

1) aplicación extendida a materiales y compuestos multicapa;
2) Una única lista positiva que incluye monómeros y otras sustancias de partida, macromoléculas obtenidas por fermentación microbiana, aditivos y auxiliares para la producción de polímeros;
3) Modificación de los simulantes usados en los ensayos de migración;
4) Incrementos de la lista de sustancias permitidas para su uso en plásticos, añadiendo sales (incluidas las sales dobles y sales ácidas) de aluminio, amonio, bario, calcio, cobalto, cobre, hierro, litio, magnesio, manganeso, potasio, sodio y zinc de los ácidos, fenoles o alcoholes autorizados;
5) Conjuntos separados de las condiciones de ensayo de la migración global y migración específica;
6) Modificación de las condiciones de tiempo y temperatura en las pruebas de migración específica.

1.5.1 Lista de sustancias permitidas

Como ya fue referido anteriormente, el Reglamento 10/2011 contiene una única lista positiva que abarca monómeros y otras sustancias de partida, macromoléculas, aditivos y auxiliares que están autorizados en la producción de polímeros. Está compuesta por aproximadamente 900 sustancias que han sido evaluadas por el antiguo Comité Científico de Alimentos (SCF) o "The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)" de la EFSA.

Esta lista, además de identificar las sustancias permitidas (número de referencia, número CAS y nombre), contiene otras informaciones, como podemos ver en la Fig. 1.2. Se trata de una lista abierta, donde se pueden añadir y eliminar sustancias o cambiar sus condiciones de uso, siempre que estas modificaciones sean soportadas por datos científicos.

1.5.2 Límite de migración específico (Specific Migration Limit - SML)

SML es la "cantidad máxima permitida de una sustancia dada liberada desde un material u objeto en alimentos o en simulantes alimentarios" (Reglamento 10/2011) y es la forma adoptada por la UE para garantizar la seguridad del consumidor.

Este límite se aplica a sustancias específicas autorizadas y se expresa en mg/kg de alimento. Se establece en base a estudios toxicológicos o de ingesta diaria tolerable (Tolerable Daily Intake - TDI), partiendo del supuesto de que una persona de 60 kg consume 1 kg de alimento diariamente durante toda su vida y ese alimento contiene el nivel máximo permitido de esa sustancia. Cuando para una sustancia no hay un SML establecido ni otras restricciones, se aplica un límite

Cuadro 1

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Nº de sustancia para MCA	Nº de ref.	Nº CAS	Nombre de la sustancia	Uso como aditivo o auxiliar de polimerización (sí/no)	Uso como monómero, otra sustancia de partida o macromolécula obtenida por fermentación microbiana (sí/no)	FRF aplicable (sí/no)	LME [mg/kg]	LME (T) [mg/kg] (nº de restricción de grupo)	Restricciones y especificaciones	Notas sobre la verificación de la conformidad
1	12310	0266309-43-7	Albúmina	No	Sí	No				
2	12340	—	Albúmina coagulada por formaldehído	No	Sí	No				
3	12375	—	Monoalcoholes alifáticos saturados lineales, primarios (C_4-C_{22})	No	Sí	No				
4	22332	—	Mezcla de (40 % p/p) 1,6-diisocianato de 2,2,4-trimetilhexano y (60 % p/p) 1,6-diisocianato de 2,4,4-trimetilhexano	No	Sí	No		(17)	1 mg/kg en el producto final expresado como grupo isocianato.	(10)
5	25360	—	Trialquil(C_5-C_{15})acetato de 2,3-epoxipropilo	No	Sí	No	ND		1 mg/kg en el producto final expresado como grupo epoxi. El peso molecular es 43 Da.	
6	25380	—	Trialquil(C_7-C_{17})acetato de vinilo	No	Sí	No	0,05			(1)
7	30370	—	Ácido acetilacético, sales	Sí	No	No				
8	30401	—	Monoglicéridos y diglicéridos de ácidos grasos, acetilados	Sí	No	No		(32)		
9	30610	—	Ácidos, C_2-C_{24}, alifáticos, lineales, monocarboxílicos, obtenidos a partir de grasas y aceites naturales, y sus ésteres con mono-, di- y triglicerol (incluidos los ácidos grasos ramificados a los niveles que se presentan naturalmente)	Sí	No	No				

Figura 1.2 *Detalle de la "Lista de la Unión de monómeros, otras sustancias de partida, macromoléculas obtenidas por fermentación microbiana, aditivos y auxiliares para la producción de polímeros"* (Reglamento no 10/2011 de la Comisión)

genérico de migración específica de 60 mg/kg, que es el valor máximo de migración total permitido (ver 1.5.3). Por la misma razón, la suma de todos SML no puede superar el valor de 60 mg/kg.

Además del SML, también existe el límite de migración específica total (Total Specific Migration Limit - SML(T)) que es el equivalente al SML, pero aplicado a un grupo de sustancias y no a sustancias individuales.

La verificación del SML se hace recurriendo a simulantes alimenticios. Estos simulantes se usan porque son matrices más sencillas que los alimentos y, consecuentemente, los análisis no están sujetos a tantas interferencias. Los simulantes también son una buena opción porque la migración en ellos debe de ser siempre superior a la que se produce en los alimentos. Esto es una ventaja desde el punto de vista de seguridad alimentaria porque nos da un valor de migración sobrestimado. Según el reglamento 10/2011, los simulantes alimenticios oficiales están especificados en la Tabla 1.2.

Las condiciones de tiempo y temperatura de las pruebas de migración están descritas en este reglamento y corresponden a las peores condiciones de contacto alimento/materiales plásticos previsibles. Estas condiciones están descritas en las Tablas 1.3 (tiempo de contacto) y 1.4 (temperatura de contacto).

Estas condiciones de ensayos de migración específica (Tablas 1.3 y 1.4) se aplican a materiales y objetos que aún no estén en contacto con alimentos. Si se trata de materiales y objetos que ya están en contacto con alimentos, se procederá a su almacenamiento según las indicaciones del fabricante (si no existen, en condiciones consideradas adecuadas a alimentos embalados). El alimento será tratado según su modo de empleo previsto (cocinado en el envase o no; partes no comestibles serán eliminadas) y será analizado para determinar la migración.

Tabla 1.2. *Lista de simulantes alimentarios según el Reglamento nº 10/2011*

Tipo de alimento	Simulante alimentario	Composición del simulante	Otras especificaciones del alimento
alimentos que tengan carácter hidrofílico y sean capaces de extraer sustancias hidrofílicas	A	Etanol 10 % (v/v)	-
	B	Ácido acético 3 % (w/v)	pH < 4.5
	C	Etanol 20 % (v/v)	alimentos con un contenido de alcohol de hasta un 20 %, y para alimentos con una cantidad importante de ingredientes orgánicos que lo hagan más lipofílico.
alimentos que tengan carácter lipofílico y sean capaces de extraer sustancias lipofílicas	D1	Etanol 50 % (v/v)	alimentos con un grado alcohólico superior al 20 % y para aceite en emulsiones acuosas
	D2 (*)	Aceite vegetal	alimentos con grasas libres en la superficie
alimentos secos	E	Poli(óxido de 2,6-difenil-p-fenileno) tamaño de partícula = 60-80 malla tamaño de poro = 200 nm	-

(*) Para alimentos descritos como "Bebidas - Diversos: alcohol etílico sin desnaturalizar" (categoría de alimentos 01.04 del Cuadro 2 del Anexo III del reglamento 10/2011), el aceite vegetal se sustituir por etanol 95%

Tabla 1.3. *Tiempos de contacto en ensayos de migración específica de materiales y objetos que aún no estén en contacto con alimentos*

Tiempo de contacto en las peores condiciones previsibles de uso	Duración del ensayo
t ≤ 5 min	5 min
5 min < t ≤ 0,5 h	0,5 hora
0,5 h < t ≤ 1 h	1 hora
1 h < t ≤ 2 h	2 horas
2 h < t ≤ 6 h	6 horas
6 h < t ≤ 24 h	24 horas
1 día < t ≤ 3 días	3 días
3 días < t ≤ 30 días	10 días
Más de 30 días	Véanse las condiciones específicas

Tabla 1.4. *Condiciones de temperatura en ensayos de migración específica de materiales y objetos que aún no estén en contacto con alimentos*

Temperatura de contacto en las peores condiciones previsibles de uso	Temperatura de ensayo
T ≤ 5 °C	5 °C
5 °C < T ≤ 20 °C	20 °C
20 °C < T ≤ 40 °C	40 °C
40 °C < T ≤ 70 °C	70 °C
70 °C < T ≤ 100 °C	100 °C o temperatura de reflujo
100 °C < T ≤ 121 °C	121 °C
121 °C < T ≤ 130 °C	130 °C
130 °C < T ≤ 150 °C	150 °C
150 °C < T < 175 °C	175 °C
T > 175 °C	Ajustar la temperatura a la temperatura real en el punto de contacto con el alimento (*)

1.5.3 Límite de migración global (Overall Migration Limit - OML)

En el Reglamento 10/2011, el OML está definido como la "cantidad máxima permitida de sustancias no volátiles liberada desde un material u objeto en simulantes alimentarios". Este límite es independiente de la naturaleza de estas sustancias y no está relacionado con los posibles efectos de estas en la salud del consumidor.

La migración global (Overall Migration – OM) máxima permitida es de 10 $mg \cdot dm^{-2}$ de superficie de contacto envase/alimento. Este límite también se puede expresar en mg/kg de alimento, siendo entonces el valor del OML igual a 60 $mg \cdot kg^{-1}$ de (se asume que 1 kg de alimento está en un envase de forma cúbica

con un área de superficie total de 6 dm^2). Esto está permitido para los siguientes casos:

- envases y otros objetos que contengan o estén destinados a contener menos de 0.5 mL o gr, o más de 10 L;
- cuando sea imposible estimar la superficie de contacto envase-alimento;
- materiales y objetos plásticos destinados a entrar en contacto con alimentos para lactantes y niños de corta edad.

La OM es determinada por gravimetría. En el caso de utilizar un simulante acuoso, después del contacto entre el material plástico y el simulante, se recoge el simulante, se evapora lentamente hasta la sequedad y se determina la masa del residuo seco. Conociendo este valor y el área de superficie del material plástico es posible calcular la OM. En el caso que se utilice un simulante graso, el método es más complejo por dos razones: 1) no es posible evaporar el simulante, por eso hay que conocer la masa del simulante antes y después del ensayo; 2) el simulante es parcialmente absorbido por el material plástico durante el ensayo, por lo tanto es necesario extraerlo y cuantificarlo posteriormente por cromatografía gaseosa (gas chromatography - GC).

La OM constituye una forma de medir la inercia del material, evita una modificación inaceptable en la composición de los alimentos y también reduce la necesidad de un mayor número de límites de migración específica o de otras restricciones. Desde el punto de vista toxicológico, la OM no aporta ninguna información importante, ya que la identidad de las sustancias que migran al alimento es desconocida, y por tanto no podemos saber cuáles son sus efectos. Estas pueden tener un efecto perjudicial o no.

1.6 Objetivos del trabajo

Como ya fue referido anteriormente, la migración es un fenómeno inevitable y que puede tener consecuencias a nivel de la salud de la población. Sin embargo, la determinación experimental de la migración suele ser bastante compleja (sobre todo debido a la diversa naturaleza de las matrices que son los alimentos), lenta y necesita grandes recursos (económicos y mano de obra), además, es imposible calcularlo para todos los alimentos, materiales y sustancias, debido a las innumerables combinaciones posibles entre ellos, y así como todas las posibles condiciones de almacenamiento.

Ante estas dificultades, surgió la necesidad de recurrir a modelos matemáticos para que la migración pueda ser estimada y, así, permitir un control práctico de los materiales destinados a entrar en contacto con alimentos.

Uno de los objectivos de esta tesis es el estudio del proceso de migración y determinación de parámetros clave (coeficiente de difusión y coeficiente de partición) necesarios para la aplicación de modelos matemáticos para la estimación de este fenómeno. Estos parámetros han sido determinados para sistemas constituidos por LDPE (aditivado con varias sustancias) en contacto directo con alimentos, siendo esto una plusvalía, pues la determinación de estos parámetros es relativamente escasa para estos casos.

Este trabajo forma parte del proyecto FACET, que ha financiado la realización de esta tesis. Esta tesis contribuyó a este proyecto con la realización de la “investigación sobre migración para obtener parámetros fundamentales de partición y de difusión que describen el proceso de migración en los alimentos envasados”. Estos datos fueron aportados al proyecto a fin “desarrollar una herramienta de modelado matemático para estimar la migración desde los materiales de envasado a los alimentos en condiciones reales de uso, con salidas

tanto determinísticas y probabilísticas para la estimación de la exposición" (FACETb).

La consecución del objetivo global pasa por el de los siguientes objetivos parciales:

1. Optimización, validación y aplicación de métodos analíticos y establecimiento de una metodología experimental para los estudios sobre la migración;
2. Preparación de ensayos de migración en un sistema compuesto por plásticos altamente aditivados con potenciales migrantes y alimentos previamente seleccionados;
3. Exhaustivo trabajo experimental destinado a la determinación de parámetros llave (coeficiente de difusión efectivo y coeficiente de partición) para estimación de migración recurriendo a modelos matemáticos debidamente validados y aceptados por la comunidad científica;
4. Evaluación del proceso de difusión frente a distintas condiciones experimentales (tiempo y temperatura), varias substancias modelo con distintas propiedades físico-químicas y diferentes alimentos con variada composición.

En esta tesis también se estudió el efecto de detergentes de lavado y aminas en muestras de biberones de policarbonato, a fin de determinar si estos productos y sustancias pueden incrementar la liberación de bisfenol A a apartir de este polímero.

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2

Time-temperature study of the kinetics of migration of benzophenone, DPBD and Uvitex® OB from LDPE films into foodstuffs

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Abstract

Migration is a mass transport process of mainly low molecular weight substances from an external source, such as plastic packaging materials, into foodstuffs. This phenomenon claims attention from the food safety point of view due to the toxicity of some plastic constituents.

This work seeks a better understanding of the migration process between food and polymeric packaging materials. Three select foods (Gouda cheese, turkey ham and orange juice), representative of different foodstuff groups, were placed in contact with low density polyethylene (LDPE) films previously additivated with benzophenone (BZP), diphenylbutadiene (DPBD) and Uvitex® OB, at three temperatures (60, 40 and 20 ºC).

After a simple extraction method with ethanol, the residual quantity of migrant in the LDPE was determined by an HPLC-DAD method that showed a good linearity within 0.05-10 $mg.L^{-1}$ range ($R^2 \geq 0.9997$). The migration key parameters, effective diffusion and partition coefficients (D_E and $K_{P/F}$, respectively), were calculated with a mathematical model based on Fick's Second Law of Diffusion. A good agreement between experimental and predicted data was achieved, meaning that this model is valid for predicting migration between food and packaging materials.

BZP was the substance that migrated more easily (it had higher D_E and lower $K_{P/F}$), while Uvitex® OB, that had the higher molecular weight and higher log P (o/w), displayed the opposite behaviour. For all migrants, the migration process was higher for the Gouda cheese, followed by turkey ham, indicating that this process would be mainly affected by the fat content of foodstuff. D_E values found in this study increased with temperature and showed a good Arrhenius relationship at the experimental temperatures. This correlation was not observed between

temperature and $K_{P/F}$ values, indicating that the partition coefficient is not significantly affected by the storage temperature.

Keywords: Migration; modelling; diffusion coefficient; partition coefficient; LDPE; food packaging;

2.1 Introduction

Packaging is an important procedure in food manufacturing and distribution, where plastics are widely used. In order to improve processing, final product properties and shelf-life, several additives are added to the plastics, such as antioxidants, plasticizers, photoinitiators, stabilizers, and whitening agents (Goulas, Anifantaki, Kolioulis and Kontominas, 2000; Sendón Garcia, Sanches Silva and Paseiro Losada, 2004; Sanches-Silva, Pastorelli, Cruz, Simoneau, Castanheira and Paseiro-Losada, 2008; Bonini, Errani, Zerbanati, Ferri and Girotti, 2008; Granda-Restrepo *et al*, 2009). These additives, as well as residual monomers, degradation products and reaction by-products, are not chemically bounded to the polymer matrix. For that reason, they are able to move freely inside the polymer and are also able to interact with food that is placed in contact with (Yam *et al*, 2009). Although these additives are usually present in low quantities, significant levels of migrants have already been found in the food in the past, raising consumers health concerns (Goulas, Anifantaki, Kolioulis and Kontominas, 2000; Grob *et al*, 2007). Low density polyethylene (LDPE), is one of the mostly used polymer in packaging materials and moreover it is where higher migration rates are usually observed. Thereby, by using LDPE in this study, a "worst case scenario" it is given (Cruz, Sanches Silva, Sendón García, Franz and Paseiro Losada, 2008).

There are three main food-packaging interactions: sorption, permeation and migration. From these phenomena the one that claims more attention from the food safety point of view is the migration due to the toxicity inherent of some plastic constituents. This process can be defined as "mass transfer from an external source into food by sub-microscopic processes" (Katan, 1996). Migration depends on several different factors: chemical and physical characteristics of the migrant and food in contact with the packaging material; packaging material;

surface/area of contact between packaging and food; time and temperature conditions (Sanchez Silva, Cruz Freire, Sendón, Franz and Paseiro Losada, 2009). This issue is extremely important and consequently specific legislation has been established by the European Union (EU). In accordance with article 3 of the Frame Regulation (EC) No. 1935/2004, food contact materials (FCM) shall not release their constituents into food in quantities which could endanger human health or bring unacceptable changes or deterioration in the organoleptic characteristics of food. A positive list of authorized substances allowed in the manufacturing of FCM, as well as their specific migration limits (SML), is specified in the Commission Regulation (EU) No 10/2011.

In order to guarantee that the FCM fulfils the requirements predicted in the legislation, migration testing performed under controlled conditions is described in the Commission Regulation (EU) No 10/2011. Verification of compliance of migration from FCM into foodstuffs shall be carried out under the most extreme conditions of time and temperature foreseeable in actual use, so the migration tests represent a “worst case scenario” as a guarantee of food safety. These tests are not easy because they are expensive, time consuming and are frequently associated with analytical/technical problems or absence of analytical methods (European Commission, 2003).

As several studies have shown, migration from plastics into a contact medium is theoretically a predictable physical process. This mass transfer, in most cases, follows Fick’s Second Law of Diffusion. This allows substituting the experimental tests by theoretical predictions of migrations values, saving time, economics and human resources (Brandsch, Mercea, Rüter, Tosa and Piringer, 2002). This new tool, migration modelling, is already included in the European legislation as an additional tool to test safety of FCM and valid mathematical models can be applied to check compliance with the legislation, if they are supported by scientific evidences that are constructed such as to overestimate real

migration. However, if the values predicted by the migration model do not fit the values allowed by the legislation, usual laboratory testing must be used to confirm migration values (Commission Regulation (EU) No 10/2011).

In the present study, three possible migrants have been chosen to study the migration process between selected foodstuffs and low density polyethylene (LDPE). The model migrants were benzophenone (BZP), 1,4-diphenyl-1,3-butadiene (DPBD) and Uvitex® OB. These substances have different applications in the polymer industry. BZP is a photoinitiator for ultraviolet (UV) cured inks that has also wetting and rheological properties that can be useful (Anderson, Castle, 2003); DPBD is a fluorescent additive and a model substance widely used in food migration studies (Sanches Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007). Uvitex® OB is an optical brightening agent, providing a brilliant bluish whitening effects, and an UV stabilizer, protecting the polymer from degradation resulting from photo-chemical processes (Quinto-Fernández, Pérez-Lamela and Simal-Gándara 2003; Sanches Silva, Sendón García, Cooper, Franz and Paseiro Losada, 2006). These model migrants have been incorporated in a LDPE film and then placed in direct contact with three foodstuffs: Gouda cheese, turkey ham and orange juice.

The aim of our work is to collect reliable data about the mass transfer process of BZP, DPBD and Uvitex® OB from LDPE into the selected foods at controlled time and temperature conditions. For migration modelling two key parameters are required: the partition coefficient ($K_{P/F}$) and diffusion coefficient (D). The data obtained from this study were used by the EU-FP7 project FACET (2008), that has the objective to develop an enhanced mathematical model to predict migration from plastic FCMs into foods under real conditions of use and thereby will help to estimate the consumer exposure to potential migrants.

2.2 Experimental

2.2.1 Foodstuff

Three food items (Gouda cheese, turkey ham and orange juice with pulp) were chosen so that each one would represent a general group of foods. Gouda cheese was selected because it is a solid foodstuff with high fat content, as well as high protein and significant water content. Turkey ham represents solid foodstuffs with high protein and water content, but low fat content. Orange juice (with suspended pulp) is an acidic and aqueous liquid food highly consumed in the European market. It has medium carbohydrates content, low viscosity and no fat content.

Gouda cheese and turkey ham samples were purchased in a local supermarket, while orange juice samples were kindly supplied by Nestlé.

2.2.2 Migrants, chemicals and standard solutions

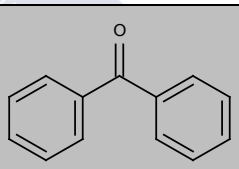
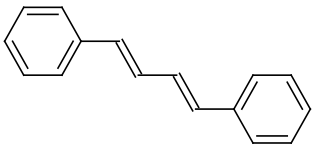
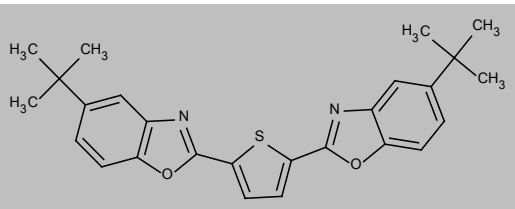
Three model migrants selected for this study were: BZP, DPBD and Uvitex® OB. BZP (purity ≥ 99 %) and DPBD (purity 98 %) were supplied by Sigma Aldrich (Steinheim, Germany), while Uvitex® OB (purity ≥ 99 %) was supplied by Fluka. Physicochemical properties and chemical structure of these migrants are summarized in Table 2.1.

Ethanol (EtOH; absolute for analysis), methanol (MeOH; for liquid chromatography), tetrahydrofuran (THF; for liquid chromatography) were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared with

a Milli-Q filter system (Millipore, Bedford, MA, USA). Sodium Azide (purity 99 %; CAS no. 26628-22-8) was supplied by Sigma Aldrich (Steinheim, Germany).

Stock solutions of each migrant were prepared with EtOH with an accurately known concentration of approximately 500 $\mu g \cdot ml^{-1}$. Calibration standard solutions containing the three model substances were prepared with EtOH. The concentration of these solutions ranged from 0.05-10 $\mu g \cdot ml^{-1}$. Solutions were stored at 4 ºC and protected from light.

Table 2.1. *Physicochemical properties and chemical structure of model migrants*

Migrant	Physicochemical properties	Chemical structure
BZP (Diphenylmethanone) Cas No.:119-61-9	MW ($g \cdot mol^{-1}$):182.2 MP (ºC): 47.8 [b] BP (ºC): 305.4 [b] Log P (o/w): 3.18 [b]	
DPBD (Trans, trans-1,4-diphenyl-1,3-butadiene) CAS No.: 886-65-7	MW ($g \cdot mol^{-1}$): 206.3 MP (ºC): 152.0 [b] BP (ºC): 350.0 [b] Log P (o/w): 4.50 [a]	
Uvitex® OB (2,5-Thiophenediylbis(5-tert-butyl-1,3-benzoxazole)) CAS No.: 7128-64-5	MW ($g \cdot mol^{-1}$): 430.6 MP (ºC): 198.5 [b] BP (ºC): 531.2 [a] Log P (o/w): 7.22 [a]	

Data obtained from Scifinder® database; a – predicted; b – experimental

MW – molecular weight; MP – melting point; BP – boiling point

2.2.3 Additivation of migrants in the plastic film

The film used as source of migrants in this study was a LDPE film (437.7±26.4 µm thick with 0.92 g cm^{-3} density) produced by Fraunhofer IVV (Freising, Germany) and additivated with BZP, DPBD and Uvitex® OB.

This additivation was performed by an immersion process. Sheets of the polymer were immersed in a highly concentrated ethanolic solution containing the three model migrants (5000 $mg \cdot L^{-1}$ of BZP; 2000 $mg \cdot L^{-1}$ of DPBD; 2000 $mg \cdot L^{-1}$ of Uvitex® OB). The container was hermetically closed and stored for 48 hours at 60 ºC. At the end of this period, the sheets were washed twice by quick immersion in fresh EtOH and dried on a kitchen paper towel for about 30 minutes.

The concentration achieved in the LDPE was 517.5±87.1 $\mu g \cdot g^{-1}$ for BZP, 797.7±82.3 $\mu g \cdot g^{-1}$ for DPBD and 809.5±36.9 $\mu g \cdot g^{-1}$ for Uvitex® OB.

2.2.4 Migration test

LDPE sheets previously additivated with the three model substances were cut in 10 cm^2 samples and accurately weighted. These polymer samples were placed in contact with the selected foodstuffs, accordingly to the food characteristics. For solid foodstuffs (Gouda cheese and turkey ham), one additivated LDPE film was placed between two previously weighted food slices (two-face contact) with the same dimension. The food/LDPE/food system was wrapped in aluminum foil and sealed in a polyamine/polyethylene bag to prevent water losses. For liquid foodstuff (orange juice), one additivated plastic sample was placed in contact with 50 ml of orange juice by immersion. To prevent microbial spoilage, a small quantity of sodium azide (approx. 1 mg) was added to the food. In the case of solid foodstuff, sodium azide was added only to the faces

that were not in contact with the LDPE sample while for the orange juice approximately 5 mg where dissolved in the liquid. The samples were storage at the following time-temperature conditions:

- 60 ºC – 0.5; 1 ; 2; 4; 8; 12; 24; 48; 96; 168 (h)
- 40 ºC – 1 ; 2; 4; 8; 12; 24; 48; 96; 168; 240 (h)
- 20 ºC – 2; 4; 8; 12; 24; 48; 96; 168; 240 (h)

Turkey ham was stored only at two temperatures, 20 and 40 ºC.

2.2.5 Extraction from LDPE

In migration studies, after contact between the polymer and the food, the quantity of migrants transferred is usually measured in the food. However, the food extraction process causes some inconvenients due to the fact that foods are complex matrixes: extraction may be very laborious and time consuming, usually there are losses related to the existence of several steps, there may be interferences and, when working with several foodstuffs, it is necessary to the develop or adapt the extraction procedure for each food. In order to avoid all these problems, a different approach was carried out. Instead of determining the quantity of migrants in the foodstuff, the migrant that remained in the polymer was determined and it was assumed that all the missing migrant was in the food.

At the end of each storing time, the LDPE films from the previous section (2.4. Migration test) were separated from food, cleaned carefully with paper tissue and placed in a flask containing 50 ml of EtOH. The flask was hermetically closed and stored in an oven at 70 ºC. After 6 hours, the flask was taken from the oven and, once room temperature was achieved, an EtOH aliquot was filtered and analysed by HPLC-DAD.

To check if any migrant still remained in the polymer matrix, the LDPE film was cleaned (by quick immersion in EtOH) to remove any migrant residues from the polymer surface and extracted a second time using the same conditions as above. Migrant residues were never detected in the second extraction solvent.

2.2.6 HPLC Chromatographic conditions

The HPLC system model 1200 (Agilent, Waldbronn, Germany) was fitted with a quaternary pump, a degassing device, an autosampler, a column thermostat system and a diode array detector (DAD). The DAD was continuously performing a scan in the range of 190 to 400 nm.

Chromatographic separation was performed with a Kromasil C18 column (250 × 3.20 mm internal diameter, 5 µm particle size) (Phenomenex, Barcelona, Spain) at 30 ºC. BZP was detected at 256 nm, DPBD was detected at 330 nm and Uvitex® OB was detected at 372 nm.

The mobile phase used in this analysis was composed of a mixture of milli-Q water and a methanolic solution of 30 % THF. During the first 4 minutes, the mobile phase was composed of a combination of 30 % milli-Q and 70 % THF methanolic solution. After this period of time, the THF methanolic solution was increased gradually until reaching 100 % at 10 min. This percentage was maintained until the end of the analysis, at 17 min. The mobile phase flow rate was 0.5 $ml \cdot min^{-1}$ during the entire time of analysis and the sample injection volume was 20 µl. For data acquisition, HP ChemStation® chromatographic software was used. The model migrants were identified by comparison of their retention times and UV spectrum with those of injected standards using the same HPLC conditions.

2.3 Results and discussion

2.3.1 HPLC method validation

The HPLC method was calibrated by using series of standard ethanolic solutions containing BZP, DPBD and Uvitex® OB. A total of ten solutions, with known concentrations ranging from 0.05-10 $mg \cdot L^{-1}$, were analyzed by triplicate. The calibration lines of concentration versus chromatographic peak areas (average of the three measurements) were obtained by linear regression. Ten samples of 1.0 $ug \cdot ml^{-1}$ were analyzed and relative standard deviations (RSD%) were calculated, ranging from 0.37 to 0.48 % for run to run precision.

Good linearity was obtained for the three model substances ($R^2 \geq 0.9997$), meaning that the method was appropriated for quantification. Parameters of each calibration line are described in Table 2.2.

Table 2.2. *Calibration line parameters*

Parameters of calibration line	Model Substance		
	BZP	**DBPD**	**Uvitex® OB**
Wavelength (nm)	256	330	372
Slope	217.29	583.97	266.55
Intercept	-1.6069	13.811	8.9436
Correlation coefficient	0.9999	0.9997	0.9999
Range ($\mu g \cdot ml^{-1}$)	0.05-10		

2.3.2 Diffusion coefficient

Experimental data obtained from the migration of BZP, DPBD and Uvitex® OB from LDPE into selected foods was fitted, by non linear regression, with the proposed model based on Arrhenius equation (Eq. 1.2 or 1.3). For this, the Solver function of the commercial software Microsoft Excel 2007® was used. The key parameters D_E and $K_{P/F}$ were calculated for each substance and food at 20, 40 and 60 ºC (except for turkey ham, where only 20 and 40 ºC were used). The values of these parameters are shown in Table 2.3.

A good fitting between the values obtained with the equation mentioned above and those obtained experimentally was found, meaning that this model can be used to predict the migration process of migrants from FCM into food. RMSE was most of the times lower or equal to 6.95 %, except for the migration of BZP into turkey ham at 20 ºC (RMSE = 10.55 %) and for the migration of DPBD into Gouda cheese at 20 ºC (RMSE = 9.75 %).

D^*_P was also calculated. This value represents a worst case situation and, as we can verify, it is always higher than D_E determined experimentally. This will be discussed in 2.3.5.

2.3.3 Migrant physicochemical characteristics

Looking the experimental D values, BZP has the highest diffusion coefficient when compared with the other two model migrants. This is most likely associated with the lower molecular weight of BZP when comparing with the other two substances, especially Uvitex® OB (Franz and Welle, 2009). DPBD has the second highest diffusion coefficient, except when is in contact turkey ham at 40 ºC. In this case, it is surpassed by Uvitex® OB. Migration of Uvitex® OB from LPDE

Table 2.3. *Partition coefficients, diffusion coefficients and RMSE values for model migrants in contact with selected foodstuffs at different temperatures; Estimated value of the polymer specific upper-bound diffusion coefficient* D^*_P

Migrant	Food	Temp (ºC)	$K_{P/F}$	D_E ($cm^2 \cdot s^{-1}$)	RMSE (%)	D^*_P ($cm^2 \cdot s^{-1}$)
BZP	Gouda cheese	60	0.41	2.12×10^{-8}	2.83	5.23×10^{-7}
		40	0.38	1.18×10^{-8}	2.89	7.04×10^{-8}
		20	0.59	2.47×10^{-9}	6.95	7.22×10^{-9}
	Turkey ham	60	-	-		-
		40	2.3	4.56×10^{-9}	1.34	7.04×10^{-8}
		20	2.0	2.44×10^{-9}	10.55	7.22×10^{-9}
	Orange Juice	60	56.9	5.95×10^{-9}	3.51	5.23×10^{-7}
		40	63.1	2.91×10^{-9}	4.58	7.04×10^{-8}
		20	48.0	1.65×10^{-9}	3.05	7.22×10^{-9}
DPBD	Gouda cheese	60	5.3	8.25×10^{-9}	5.21	3.86×10^{-7}
		40	5.6	4.27×10^{-9}	5.59	5.21×10^{-8}
		20	4.8	6.90×10^{-10}	9.75	5.34×10^{-9}
	Turkey ham	60	-	-	-	3.86×10^{-7}
		40	23.6	2.84×10^{-10}	2.96	5.21×10^{-8}
		20	34.2	1.05×10^{-10}	0.92	5.34×10^{-9}
	Orange Juice	60	538.6	4.44×10^{-11}	1.30	3.86×10^{-7}
		40	520.9	1.94×10^{-11}	1.68	5.21×10^{-8}
		20	795.4	1.15×10^{-11}	1.32	5.34×10^{-9}
Uvitex® OB	Gouda cheese	60	8.8	2.78×10^{-9}	3.67	3.82×10^{-8}
		40	9.3	7.92×10^{-10}	2.07	5.15×10^{-9}
		20	12.7	1.86×10^{-10}	3.93	5.28×10^{-10}
	Turkey ham	60	-	-	-	3.82×10^{-8}
		40	47.8	3.38×10^{-10}	2.53	5.15×10^{-9}
		20	27.6	4.48×10^{-11}	3.67	5.28×10^{-10}
	Orange Juice	60	> 1000	2.04×10^{-12}	3.11	3.82×10^{-8}
		40	> 1000	4.24×10^{-12}	3.76	5.15×10^{-9}
		20	> 1000	3.72×10^{-12}	1.68	5.28×10^{-10}

is very low for orange juice. In Fig. 2.1, a comparison of the migration of the three model substances into Gouda cheese is represented.

Most the results obtained showed that diffusion rates decrease with the increment of molecular weight of migrants. This is observed in many other studies, where MW is considered a critical factor for the diffusion coefficient (Sanches-Silva, Pastorelli, Cruz, Simoneau, Castanheira and Paseiro-Losada, 2008; Reynier, Dole and Feigenbaum, 2002; Helmroth, Rijk, Dekker and Jongen, 2002), while other factors are usually not referred. The MW differences between BZP and DPBD are not very different (182.2 and 206.3, respectively), so this would imply that, if the MW was the only physicochemical characteristic of the migrant affecting the diffusion coefficient, BZP and DPBD should have similar D_E values for the same food and temperature (as it happens for D^*_P where the MW is the only migrant characteristic taken in account, for a given temperature and polymer). Nevertheless, although MW of Uvitex® OB is much higher (MW = 430.6), D_E values obtained for DPBD are sometimes closer to the D_E values obtained from Uvitex® OB rather than the ones obtained for BZP. This indicates that other physicochemical characteristics are affecting this parameter. Some works investigated other properties that, together with the MW, may interfere with the diffusion coefficient: polarity, volatility, functional groups, molecular size, shape/structure and volume (Franz and Welle, 2009; Helmroth, Dekker and Hankemeier, 2002; Widén, Leufvén and Nielsen, 2004; Reynier, Dole, Feigenbaum and Feigenbaum, 1999; Stoffers, Störmer, Bradley, Brandsch, Cooper, Linssen and Franz, 2004; Brandsch, Mercea, Rüter, Tosa and Piringer, 2002).

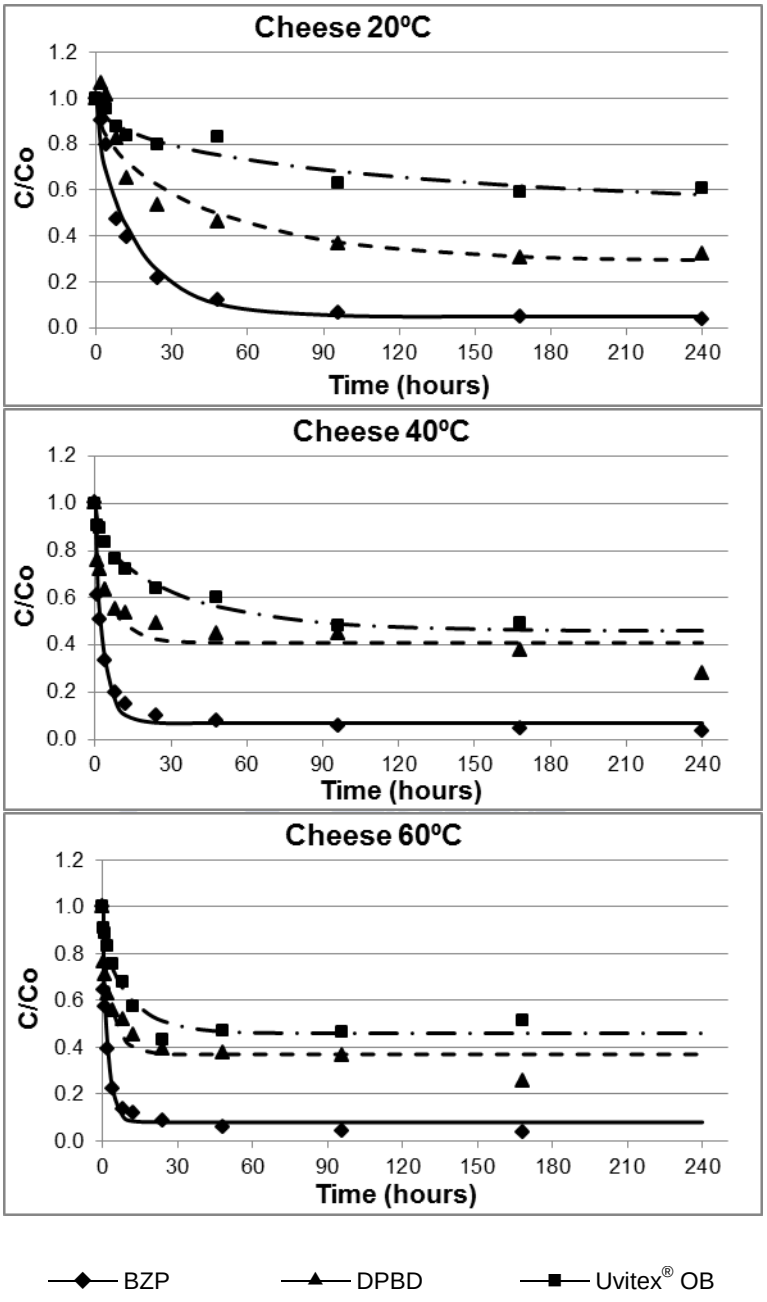


Figure 2.1. *Migration of BZP, DPBD and Uvitex® OB from LDPE into Gouda cheese, at 20, 40 and 60 ºC*

2.3.4 Foodstuff physicochemical characteristics

Fat content is one of the most important factors affecting migration of substances from FCM into foodstuffs. Fats are able to induce polymer swelling or leaching migrants (because they are usually lipophilic) and, consequently, are able to increase the transfer of substances into foodstuffs (Riquet, Wolff, Laoubi, Vergnaud and Feigenbaum, 1998; Sanches Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007).

Generally, for each one of the substances studied, the D_E values were proportional to the food fat content. Consequently, D_E was higher for Gouda cheese (27 % fat content), followed by turkey ham (2 % fat content) (USDA database, 2011) and, lastly orange juice (<0.5 % fat content) (food's nutritional information) (Fig. 2.2).

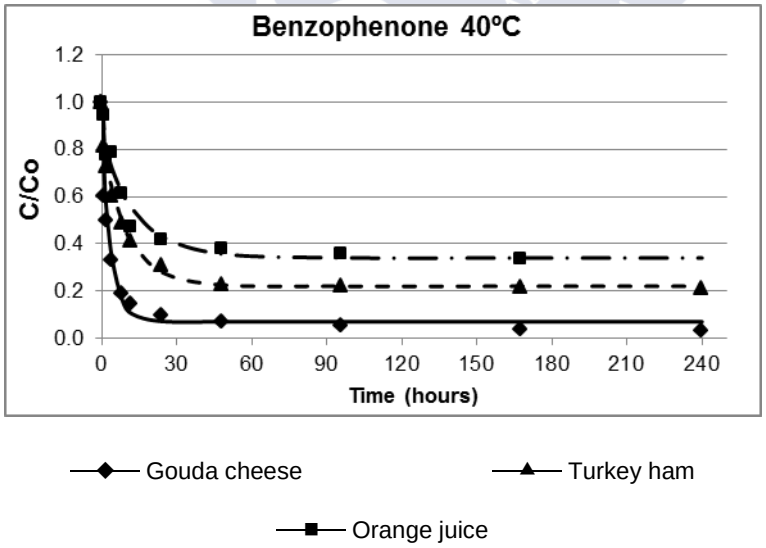


Figure 2.2. *Migration of BZP from LDPE into Gouda cheese, turkey ham and orange juice, at 40 ºC; comparison between selected foodstuffs*

2.3.5 Diffusion coefficient linearity

Besides depending on the migrant and food characteristics, D_E also depends on the temperature, being higher when the temperature increases. To check diffusion coefficient linearity in the range of 20 - 60 ºC, eq. 1.5 was used.

The D_0, E_A and R^2 obtained from the Arrhenius equation are represented in Table 2.4. The correlation coefficients of the Eq. 1.5 were always ≥ 0.9492, confirming that, using this equation it is possible to predict D_E for any temperature in the range of 20 and 60 ºC. The only case where the correlation coefficient was lower was for Uvitex® OB in orange juice (R^2 = 0.6262). This may be explained by the fact that, in this specific case, migration was very low. In this situation, minimal experimental variations (e.g.: differences in food samples, variations in film thickness, dispersion of the migrant in the polymer) may induce higher error when estimating the migration parameters. In this same Table, it is also possible to observe the D_0 and E_A for the diffusion coefficients predicted by Piringer equation (D^*_P).

Using Eq. 1.5, it is possible to state that, even that D^*_P is intended to represents a worst case scenario, at some temperature D_E is higher than D^*_P (Fig. 2.3). The reason is that, using Piringer prediction values on Eq. 1.5, the E_A (and consequently the slope of Eq. 1.5) is constant (86.9 $kJ \cdot mol^{-1} \cdot K^{-1}$). As a result, unless experimental E_A and predicted E_A values were exactly equal, the condition $D^*_P > D_E$ for $0 < T\ (K) < \infty$ would never be fulfilled (assuming that linearity would remain for every temperature and no changes resulting from temperature are observed in the food, polymer or migrants).

The temperatures where $D_E > D^*_P$ are represented in the last column of Table 2.4. For all cases, D_E was lower than D^*_P for the experimental temperatures (20 - 60 ºC), meaning that D^*_P represents a worst case scenario for this

temperature range. Nevertheless, assuming that diffusion coefficient linearity would remain for all temperatures, at some point, D_E will surpass D^*_P. In some cases, this happens at very low and impracticable temperatures (-24.3 to -91.6 ºC) so we may assume that, for a real use case scenario, D^*_P clearly represents a worst case scenario. In other cases, this happens at higher temperatures (3.3 to 8.2 ºC), indicating that, in real use cases, the diffusion coefficient estimated with Piringer equation is lower than the real one and, consequently, the real migration might be higher than the predicted with Eq. 1.5.

Table 2.4. *Pre-exponential factor and activation energy; Comparison between experimental and predicted values*

Migrant	Food	Experimental			Piringer prediction		Min. T (ºC) for $D > D^*_P$ [a]
		D_0 ($cm^2 \cdot s^{-1}$)	E_A ($kJ \cdot mol^{-1}$)	R^2	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($kJ \cdot mol^{-1}$)	
BZP	Gouda cheese	0.195	44.0	0.9522	2.22×10^{7}	86.9	5.1
	Turkey ham	4.50×10^{-05}	23.9	1.0000			8.2
	Orange Juice	6.49×10^{-05}	25.9	0.9890			3.3
DPBD	Gouda cheese	0.943	50.8	0.9492	1.64×10^{7}	86.9	-13.0
	Turkey ham	6.00×10^{-04}	37.9	1.0000			-27.9
	Orange Juice	7.74×10^{-07}	27.2	0.9727			-39.2
Uvitex® OB	Gouda cheese	1.12	54.9	1.0000	1.62×10^{6}	86.9	-1.5
	Turkey ham	2.49×10^{03}	77.1	1.0000			-91.6
	Orange Juice	3.97×10^{-10}	12.5	0.6262			-24.3

[a] assuming that linearity would remain out of the experimental temperatures range

2.3.6 Partition coefficient

The partition coefficient ($K_{P/F}$), i.e. the thermodynamic equilibrium of the migration process, is defined as the ratio of migrant concentration in the polymeric

material and in the food phase, at equilibrium. Contrary to the diffusion coefficient and accordingly to Gandek *et al* (1989), $K_{P/F}$ is not significantly affected by the temperature.

In this study, the migrant concentration in the foodstuff was not determined directly. Instead, it was assumed that all the substance that left the polymer is present in the food, i.e., the concentration of the migrants in the food at equilibrium is equal to the difference of concentrations determined in the LDPE at t=0 and at the equilibrium state. This allowed avoiding extraction of the migrants from the food that is a laborious process and requires a great amount of time and economics.

Similarly to D_E, the $K_{P/F}$ also depends on the polarity and solubility of the migrant in the food (Tehrany, Mouawad and Desobry, 2007). Lower $K_{P/F}$ values (higher quantity of migrant leaving the polymer into the foodstuff) were found for BZP, while higher $K_{P/F}$ values were found for Uvitex® OB. By comparing the experimental $K_{P/F}$ values and the log P (o/w) (Table 2.1) of each migrant, both values could be related: the substances where the highest $K_{P/F}$ (Uvitex® OB) was found is the one with the highest log P (o/w), while the one with the lowest $K_{P/F}$ (BZP) has the lowest log P (o/w). From the food point of view lower $K_{P/F}$ were found for Gouda cheese. Orange juice revealed the highest $K_{P/F}$ values between these three foodstuffs.

The lowest $K_{P/F}$ obtained was for BZP in contact with Gouda cheese ($K_{P/F} \leq 0.59$). This means that, when getting close to the equilibrium state, around 95 % of the BZP is in the Gouda cheese because this migrant is highly soluble in this foodstuff (due to the high fat content). The opposite situation was found for Uvitex® OB in contact with orange juice, where the amount of this model substance that migrated into the foodstuff was very low ($K_{P/F} > 1000$).

Once the $K_{P/F}$ values were analysed, variations between the same food-migrant combination at different temperatures and even between the duplicates were observed. This could be caused by errors inherent to the experimental method, polymer thickness variation, small differences within the food matrix and/or migrant was not homogeneously distributed in the polymer. Other reason for this is the fact that, in some cases, a perfect fit between the experimental data and the proposed mathematical model (Eq. 1.2 and 1.3) was not found or there was not enough time to achieve equilibrium, contributing to some differences between predicted and real $K_{P/F}$ value. As such, these values should only be regarded as indicative.

2.4 Conclusion

In this study, reliable data regarding the migration of BZP, DPBD and Uvitex® OB from LDPE into Gouda cheese, turkey ham and orange juice were attained with the purpose of better understand the mass transfer process of migrants in plastic package-food systems. Key parameters for migration model were estimated using a simplified mathematical model based on Fick's Second Law of Diffusion. A good correlation between experimental and modelled values was found. So this model would appropriated for migration estimation and would allow a reduction in the number of analyses required for testing plastic FCM compliance with current legislation, saving time and economical resources.

The results obtained in this work confirmed that several parameters have a deep influence in the migration behaviour. The storage temperature affected greatly the diffusion coefficient, while the partition coefficient was not significantly

affected. The diffusion coefficients for each migrant in each LDPE-food system showed a good Arrhenius relationship, allowing the estimation of D_E for any temperature in the experimental temperature range (20 - 60 ºC).

BZP, which is the migrant with lower MW and more volatile, showed a higher migration potential (higher D_E and lower $K_{P/F}$). On the other hand, Uvitex® OB, having the highest MW, showed the lowest migration potential (lower D_E and higher $K_{P/F}$).

It was also observed that the foodstuff physicochemical characteristics also affected the mass transfer processes. Migration was higher in Gouda cheese, followed by turkey ham and finally by orange juice. The feature responsible for this behaviour appears to be mainly the fat content of the food, although other factors may also be contributing for it.

The results of this study are meant to be used by the EU-FP7 project FACET to develop an enhanced mathematical modelling tool to predict the migration from FCM into food under real use conditions and to predict consumer's exposure to substances in foodstuffs taken from FCM.

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Methodology to determine diffusion and partition coefficients of Triclosan from LDPE into selected foods by direct contact

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Abstract

Plastics are one of the most widely used food contact material (FCM). In order to improve plastics properties or to facilitate their processing, additives are commonly employed. Nevertheless, due to the nature of polymeric matrices, these additives and other low molecular weight molecules (residual monomers and oligomers) are able to move freely within the polymeric structure, allowing food-packaging interactions such as migration. Migration is a phenomenon where mainly low molecular weight substances initially present in the packaging material migrate into the foodstuff and, due to the toxic effect of some additives, it may represent a threat for consumer's health.

Due to the fact that the information about migration from packaging materials to real food matrices is very scarce, this work objective's is to improve the understanding of this transfer process between plastic packaging and food. For this, a low density polyethylene film highly additivated with triclosan (a substance with biocide properties) was placed in contact with different foodstuffs, at different temperatures. The selected foodstuffs were Gouda cheese, turkey ham, cooked ham, pâté, orange juice, red wine and tomato sauce.

After being in contact with foodstuff, the model migrant was extracted with ethanol and quantified by a HPLC-DAD method. Two vital parameters for migration modelling (effective diffusion coefficient D_E and partition coefficient $K_{P/F}$) were calculated according to an equation based on the Fick's Second Law of Diffusion. A good fitting between experimental and predicted data was achieved, confirming that the suggested model is appropriated for predicting migration in packaging-food systems.

The migration process was greatly affected by the foodstuff used and by the storing temperature. Regarding the temperature used, the migration of triclosan

was higher (high D_E and low $K_{P/F}$) when the tested foodstuff was rich in fat. The storing temperature affected the D_E, being higher when the system food/LDPE/food was stored at 60 ºC, while the $K_{P/F}$ was not significantly affected by this parameter. A good linearity was found between the D_E values obtained at different temperatures, allowing estimating D_E for any temperature within the experimental temperature range.

Keywords: Migration modelling; diffusion; partition; Triclosan; food packaging

3.1 Introduction

Food packaging is a procedure of extreme importance in food industry that prevents food from getting damaged or spoiled during the length of time it is stored and/or distributed to the consumer. (Sanches Silva, Cruz, Sendón García and Paseiro-Losada, 2007). A wide variety of packaging materials may be found: plastic, paper, paperboard, metal and glass. These materials are chosen in order to better please the consumer and industry demands. As such they shall be cheap, suitable specific applications and easily processed. Also any material or article designed to be in direct or indirect contact with foodstuff must be sufficiently inert to prevent substances from being transferred to food in quantities large enough to endanger human health, to bring unacceptable changes in the composition of the food or to provoke deterioration of organoleptic properties (Skjevrak *et al*, 2005; Canellas, Aznar, Nerín and Mercea, 2010).

Polymers are not an inert material and are able to interact with the surrounding environment, allowing food-packaging interactions: sorption, permeation and migration (Risch, 2000). Permeation involves the exchange of substances between the environment and the foodstuff through the packaging material (Del-Valle, Almenar, Hernández-Muñoz, Lagarón, Catala and Gavara, 2004). Sorption leads to aroma and flavour losses due to the transfer of substances from foodstuff into packaging, while migration is defined as a diffusion process of mainly low weight substances initially present in the packaging material into foodstuff (Sanches Silva, Cruz Freire, Franz and Paseiro Losada, 2007). From these three phenomena, migration is the one that raises more concern from the human health point of view, because substances that migrate from plastic films into foodstuff are potentially harmful for the consumer (Poças and Hogg, 2007). This happens because plastic additives (commonly added to the plastic to improve their characteristics), residual monomers and oligomers (resulting from an

incomplete polymerization process), are not chemically bounded to the polymer matrix and are able to move freely within it (Sanches Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007). Even so, plastics are widely employed as packaging materials in direct contact with food and its use keeps rising considerably.

In order to check the quality of the materials intended to come into contact with foodstuffs, a group of rules and conditions to regulate the migration of constituents of these materials are described in the current legislation (Reglamento (UE) Nº 10/2011). In this directive, due to the complexity of the real food matrices, the substitution of those by food simulants is authorized, according to the nature of the food (aqueous, fatty, acidic and alcoholic foods). Also, strict time and temperature conditions for the migration tests are defined.

Migration tests are usually not an easy task. Besides being complex from the analytical point of view (due to the complexity of the food matrixes), they involve a great number of analysis and consequently a large amount of time, money and availability of qualified technicians (Sanches Silva, Cruz, Sendón García, Franz and Paseiro Losada, 2007; Piringer, 1994). One solution to decrease the number of migration tests is the use of mathematical models that enables to predict the migration process, because this mass transfer from plastic film into foodstuff is a predictable physical process that obeys in most cases to Fick's Second Law of Diffusion (Brandsch, Mercea, Tosa and Piringer, 2002).

Mathematical migration modelling is already contemplated as a tool to ensure FCM quality in the Commission Regulation (EU) no. 10/2011. According to this regulation, the value of a specific migration of the substance can be determined by an adequate experimentation or by the application of accepted diffusion models based on scientific evidence which can be applied to test for compliance with existing regulations. If non-compliance is demonstrated by

migration modelling, usual experimental methods must be used to confirm the predicted migration data. The relevance of the estimation of migration through mathematical models has already been referred in several previous works (Sanches Silva, Cruz, Sendón García, Paseiro-Losada, 2007; Duffy, Hearty, Mccarthy and Gibney, 2007; Begley *et al*., 2005; Petersen, Trier and Fabech, 2005; Franz, 2005; Brandsch, Mercea, Ruter, Tosa and Piringer, 2002)

For migration modelling, two indispensable parameters are needed: the diffusion coefficient (D) that is related with the kinetic of the process, measuring the rate at which the model substance migrates into the FCM; and partition coefficient ($K_{P/F}$), defined as the ratio of migrant concentration in the polymeric material and its concentration in the food at equilibrium, representing the thermodynamic equilibrium. (Granda-Restrepo *et al*, 2009; Tehrany and Desobry, 2004)

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; CAS no. 3380-34-5) is a white crystalline powder with high lipophilicity (log P (o/w) = 4.76) (Bones, Thomas, Nesterenko and Paull, 2006). This substance possesses antibacterial properties and also has some antifungal and antiviral properties. Being active against several types of Gram-positive and Gram-negative bacteria, it is bacteriostatic at low concentrations but when the concentration increases it becomes bactericidal. (Russel, 2004; Sanches-Silva, Sendón-García, López-Hernández and Paseiro-Losada, 2004). According to McMurry *et al.* (1998), triclosan works by irreversibly inhibiting the active site of the enoyl-acyl carrier protein redutase enzyme (humans do not have this enzyme), preventing the bacteria from synthesizing fatty acid and, consequently, from building cell membranes and from reproducing.

In this work, framed in the EU project FACET (2008), the migration kinetic of triclosan from low density polyethylene (LDPE) into real foodstuffs (Gouda cheese,

cooked ham, turkey ham, tomato sauce, pâté, orange juice and red wine) was studied at different storing conditions. These foodstuffs were selected so that each one would represent a different foodstuff group according to their physicochemical characteristics: physical state, viscosity, fat/water ratio, acidity and fat, protein and hydrocarbon contents. LDPE was the polymer chosen because, amongst the plastics films, this is one of the most used and, by having the highest diffusion rates, represents a worst case scenario. (Cruz, Sanchez Silva, Sendón García, Franz and Paseiro Losada, 2008). Key parameters of migration (effective diffusion and partition coefficients) were determined by fitting the experimental data with a proposed model based on Fick's Second Law of Diffusion. The results are meant to be used by the FACET project to develop a software for deterministic and probabilistic modelling of food chemical intake.

3.2 Materials and methods

3.2.1 Plastic film

A LDPE film produced by GAIKER Technology Centre (Zamudio, Spain) was used in this study as a model migrant source for the mass transport tests. This film had a thickness of 395.4±17.6 nm and a density of 0.91 g cm^{-3}. The additivation of the plastic was done during production of the film by an extrusion process.

The initial concentration of triclosan in the LDPE film was 1275.6±131.2 $\mu g \cdot g^{-1}$.

3.2.2 Chemicals and standard solutions

Ethanol (EtOH; absolute for analysis), acetonitrile (ACN; for liquid chromatography) were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA, USA). Sodium azide (purity 99%; CAS no. 26628-22-8) was supplied by Sigma Aldrich (Steinheim, Germany).

For this study, triclosan (CAS no. 3380-34-5) was chosen as model migrant. This substance was supplied by Fluka and has a purity ≥ 97.0 %. Triclosan is an additive with biocide characteristics that may be used to prevent microbial growth in the surface of plastics intended to be used as food packaging material. Its physicochemical characteristics are: molecular weight (MW) = 289.54; boiling point (BP) = 344.6 ºC; melting point (MP) = 54-57.3 °C; log P (o/w) = 4.76 (data acquired from Scifinder® database). Triclosan molecular formula is represented on Fig. 3.1.

A stock solution of the model migrant triclosan was prepared in EtOH with an accurately known concentration of approximately 1000 $\mu g \cdot ml^{-1}$. From this stock solution, an intermediate solution of 100 $\mu g \cdot ml^{-1}$ was prepared. Calibration standard solutions containing triclosan were prepared through successive dilutions with EtOH. The concentration of these solutions ranged from 0.05-10 $\mu g \cdot ml^{-1}$. Solutions were stored at 4 ºC and protected from light.

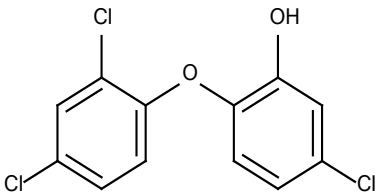

Figure 3.1. *Triclosan chemical structure*

3.2.3 Food samples

For this experiment, a total of seven food samples were chosen: Gouda cheese, turkey ham, cooked ham, pâté, tomato sauce, orange juice and red wine. These foodstuffs were chosen so that each one would be representative of a group of foods (Table 3.1). Some of these foods, Gouda cheese, turkey ham, cooked ham and red wine were bought in a local supermarket, while pâté, tomato sauce and orange juice were kindly supplied by Nestlé. A total of ten samples were arranged for each kinetic curve for a given foodstuff and temperature. A total of three temperatures were selected for this study: 20, 40 and 60 ºC (except for turkey where only the two lower temperatures were used). At each predetermined time (Table 3.1), a sample was taken and analysed as explained below. Duplicates were always made for each kinetic curve.

3.2.4 Migration test

Samples were prepared differently according to their physical state. Therefore, they were separated in three groups: liquid food (orange juice, red wine and tomato sauce); solid food (Gouda cheese, turkey ham and cooked ham); and pâté, that was a semi-solid food with high viscosity**.**

Liquid food: 50 ml of liquid food were placed in a 60 ml amber flask. To prevent microbial spoilage during storage, a small quantity of sodium azide (approx. 5 mg) was diluted in the food sample. A polymer sample measuring 10 cm^2 and previously weighted was then placed in contact with the food sample by immersion. The amber flask was tightly closed to prevent water loss during storage.

Solid food: food slices were cut in two 10 cm^2 samples and accurately weighted. A LDPE film sample with the same dimensions (previously weighted) was taken and placed between the two food samples. Sodium azide (approx. 1 mg) was added to food side that is not in direct contact with the LDPE film. The system foodstuff/LDPE/foodstuff was slightly compressed (just enough to ensure intimate and uniform contact in all the contact area between foodstuff and polymer), wrapped in aluminium foil and sealed in a polyamine/polyethylene (PA/PE) bag. The PA/PE was meant to prevent water loss, meanwhile the aluminium foil was applied to prevent mass transfer phenomena between the foodstuff/LDPE/foodstuff system and the PA/PE bag.

Pâté: 20 g of pâté were placed in a 30 ml amber flask together with sodium azide (approx. 5 mg). A polymer sample measuring 2x5 cm^2 and accurately weighted was shaped like a ring and immersed in the food sample. This was meant to decrease the quantity of pâté in contact with the additivated LDPE film. The amber flask was tightly closed to prevent the foodstuff from drying.

To prepare a migration curve, a total of ten samples were taken of each foodstuff for each storage temperature. After the several foodstuffs have being properly placed in direct contact with the LDPE additivated with triclosan, each food item was storage at three different temperatures (20, 40 and 60 ºC), except for turkey ham that was storage only at 20 and 40 ºC. The storage times are represented in Table 3.1.

At the end of each storage time (see Table 3.1), a sample was taken from the laboratory oven. The LDPE film was separated from the foodstuff and carefully cleaned with a paper tissue. When properly cleaned, the polymer was extracted with 50 ml of EtOH at 60 ºC. After 6 hours, the flask was taken out of the oven, an EtOH aliquot was filtered with a 0.45 µm filter and analysed by HPLC.

Table 3.1. *Foodstuff brief description and composition. Experimental storage temperature-time conditions*

Food	General description	Fat (%)	Protein (%)	CH (%)	Water (%)	Fat/Water ratio	Storage conditions temp. (ºC) and time (h)
Gouda cheese[a]	Solid food with high fat and protein content.	27.4	24.9	2.2	41.5	0.66	20 ºC: 2; 4; 8; 12; 24; 48; 96; 168; 240; 360 40 ºC: 1; 2; 4; 8; 12; 24; 48; 96; 168; 240 60 ºC: 0.5; 1; 2; 4; 8; 12; 24; 48; 96; 168
Turkey ham[a]	Solid food with high water and protein contents and low fat content.	1.8	21.0	1.7	72.0	0.025	
Cooked ham[a]	Solid food with high water and protein contents and medium fat content.	4.0	17.9	0.2	73.5	0.11	
Pâté[b]	Semi-solid food with high viscosity and high fat content.	23.0	10.0	3.5	61.1	0.38	
Tomato sauce[b]	Liquid food with high water content and very low protein and fat content.	0.2	1.5	4.6	NDA	NDA	
Orange juice[b]	Acidic aqueous liquid food with pulp and high CH content. Extremely low fat content.	< 0.5	< 1.0	9.0	87.2	< 0.0057	
Red wine[a]	Aqueous liquid food with ethanolic content. No fat content	0.00	0.07	2.6	86.5	0.00	

[a] data acquired from USDA database; [b] data acquired from food package nutritional information

CH – carbohydrates; NDA – no data available

In order to verify if the extraction had been complete, the plastic film was cleaned with EtOH to remove any remaining triclosan that came from the ethanolic extraction solution and it was submitted to a second extraction under the same conditions.

3.2.5 HPLC Chromatographic conditions

The HPLC system model 1200 (Agilent, Waldbronn, Germany) was equipped with a quaternary pump, a degassing device, an autosampler, a column thermostat system and a diode array detector (DAD). The DAD was continuously performing a scan in the range of 190 to 400 nm. For data acquirement, the Chemstation chromatographic software was used.

Chromatographic separation was achieved with a Kromasil C18 column (250 × 3.20 mm internal diameter, 5 µm particle size) (Phenomenex, Barcelona, Spain), thermostatized at 30 ºC.

A gradient elution method was applied. The mobile phase used in this analysis was composed by a mixture of milli-Q water and ACN. During the first 3 minutes, the mobile phase was 40 % milli-Q/60 % ACN, after which the ACN would increase gradually until reaching 100 % at 9 min. The total run time of the analysis was 17 min to clean the column. The mobile phase flow rate was 0.5 $ml \cdot min^{-1}$ during the entire time of analysis and the sample injection volume was 10 µl. Triclosan was detected at 256 nm and it was identified by comparing its retention time and UV spectra with those of injected standards using the same HPLC conditions.

3.3 Results and discussion

3.3.1 Sample extraction

Performing migration studies between packaging and foodstuff (or food simulant) may reveal a very complex and laborious task. The main reason for this is that the quantity of migrant that was transferred between the package material and food is determined after performing the extraction of the migrant from the food. These foods represent a very complex matrix which often leads to analytical and technical difficulties (Sanches Silva, Cruz, Sendón García and Paseiro-Losada, 2007). In this study, in order to bypass these adversities, a different approach was taken in order to simplify the experimental work. For this, the quantity of the model substance that migrated was not measured directly in the foodstuff. Instead, it was determined by measuring the difference of the quantity of migrant in the LDPE additivated film before and after being placed in contact with the selected foodstuffs.

By performing this, it was possible to rapidly test a much larger number of samples simultaneously. Also, it allowed to greatly reducing the workload and costs that are usually required for these analyses.

3.3.2 Migration kinetics of triclosan from LDPE films into selected foodstuffs

Migration of a substance from FCM into foodstuff is a complex mass transfer process that depends on several factors such as time, temperature, polymer type and food physicochemical characteristics, particularly the fat content. Even so, this is a predictable physical process (Ossberger, 2009). The relative amount of

triclosan that was transferred from the LDPE into the foodstuff is represented in Fig. 3.2. As it can be observed in this figure, triclosan migrates almost completely into the foods with higher fat content (Gouda cheese and pâté) and it is lower when the fat content decreases. Red wine, followed by tomato sauce and orange juice, is where the fat content is lower and, consequently, lower amounts of triclosan are transferred into the foodstuff.

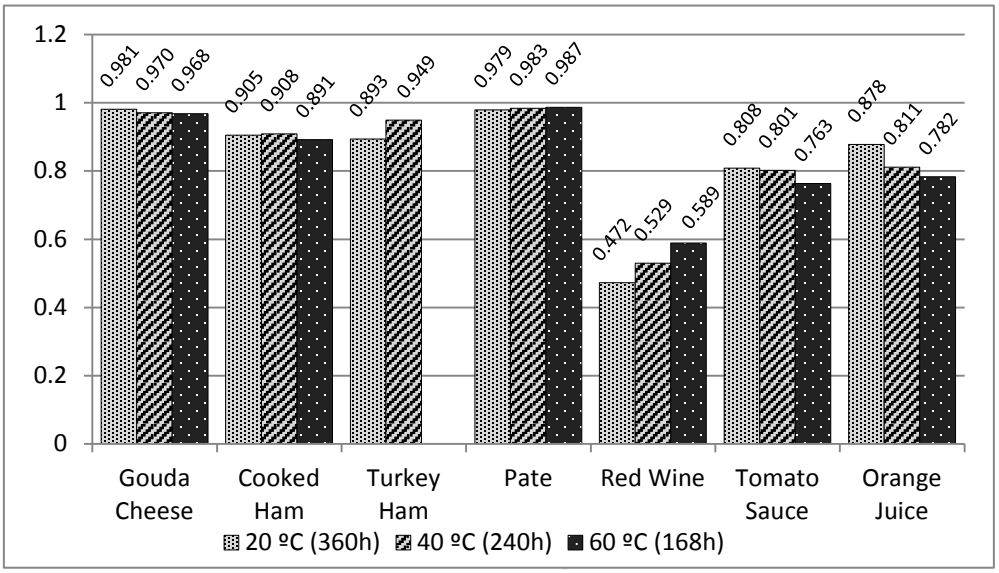

Figure 3.2. *Ratio between amount of triclosan that migrated into foodstuff and initial amount in the LDPE film*

With the proposed mathematical model (Eq. 1.2 and 1.3), the migration parameters D_E and $K_{P/F}$ where successfully calculated and are shown in Table 3.2. The RMSE values were also calculated and were low, ranging between 1.7 and 4.7 % for all selected foodstuff and temperatures, meaning that there was a good correlation between experimental and estimated data. The highest value (4.7 %)

was found for orange juice at 40 ºC, while the lowest (1.7 %) was found for red wine at 20 ºC.

Table 3.2. *Calculated D, $K_{P/F}$ and RMSE values for the migration of triclosan from additivated LDPE films into foodstuffs*

Food	Temp. (ºC)	D_E ($cm^2.s^{-1}$)	$K_{P/F}$	RMSE (%)
Gouda Cheese	60	2.05×10^{-8}	0.53	3.5
	40	1.08×10^{-8}	0.74	1.9
	20	1.49×10^{-9}	0.51	4.5
Turkey Ham	60	-	-	-
	40	6.20×10^{-9}	1.0	3.2
	20	9.42×10^{-10}	0.91	1.8
Cooked Ham	60	2.89×10^{-9}	1.7	3.1
	40	2.41×10^{-9}	1.5	3.2
	20	6.91×10^{-10}	1.2	1.9
Pâté	60	2.31×10^{-8}	1.1	3.3
	40	8.26×10^{-9}	1.4	2.5
	20	1.40×10^{-9}	3.0	2.6
Tomato Sauce	60	1.67×10^{-9}	58.2	3.4
	40	1.10×10^{-9}	44.9	4.3
	20	5.42×10^{-10}	32.5	3.1
Orange Juice	60	4.02×10^{-9}	41.7	4.0
	40	2.19×10^{-9}	25.1	4.7
	20	6.50×10^{-10}	15.9	2.3
Red Wine	60	3.84×10^{-9}	88.4	2.7
	40	2.27×10^{-9}	119.3	3.2
	20	8.74×10^{-10}	163.0	1.7

3.3.3 Diffusion coefficient

The effective diffusion coefficient is the parameter that describes the kinetics of the migration process and is greatly affected by the temperature and by the properties of the migrants, foodstuffs and polymers under study.

By comparing the D_E values obtained for each food (see Table 3.2), the foods with higher D_E are the Gouda cheese and pâté. This is not surprising because, from the selected foods, these are the ones with higher fat content. The fat content is one of the factors linked to the food that most affects the migration process. Not only is responsible for increasing the amount of migrant that is transferred from the FCM into the food (lower $K_{P/F}$), but also affects the kinetics of this process, by incrementing the rate of the migration, which is reflected in a higher D_E (Sanches Silva, Cruz, Sendón García and Paseiro-Losada, 2007).

By looking into the D_E values attained for the different foods at different temperatures, D_E clearly increases with the temperature. To check linearity between the experimental D_E values, equation 1.5 was used:

A good linear correlation between the D_E of the different temperatures of each foodstuff was found. This means that, after calculating the E_A and D_0 for each foodstuff, these equations can be used to determine D_E for any temperature between 20 and 60 ºC. The R^2 values were always higher than 0.9390, except for the cooked ham where $R^2 = 0.8705$. The most probable justification for this is the fact that, from all the selected foodstuffs, cooked ham is the one that has higher heterogeneity between the different samples. Even though, these samples were chosen so that they were the most similar possible (at naked eye), that was a very difficult task and it was not enough to eliminate this source of errors. The D_0, E_A and R^2 obtained from the equation 6 are represented in Table 3.3.

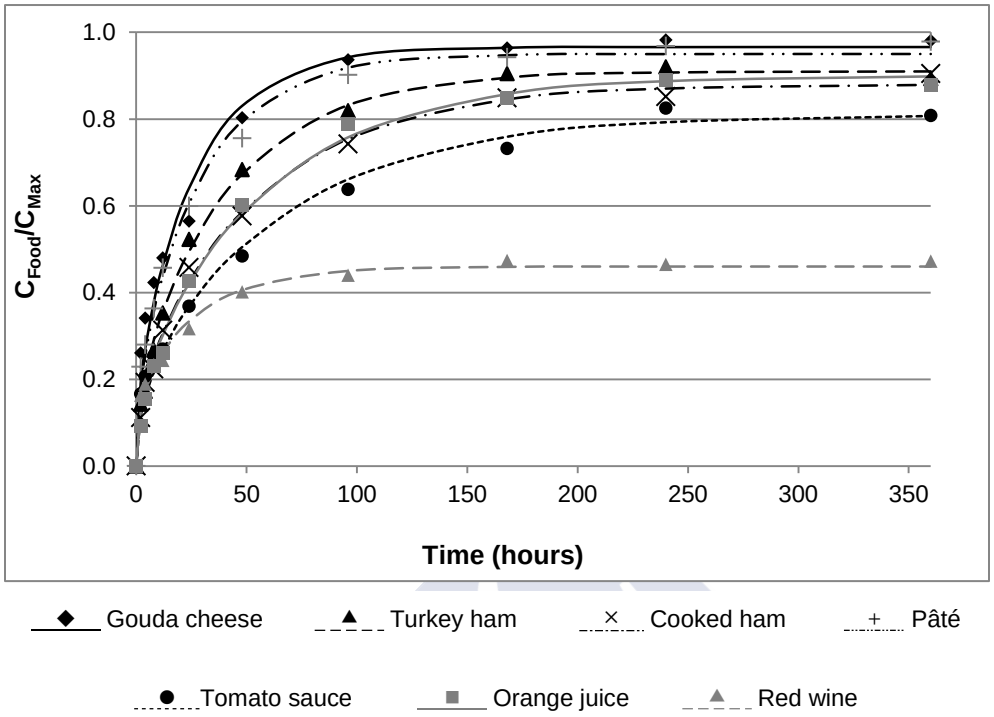

Figure 3.3. *Migration of triclosan from additivated LDPE into selected foodstuffs, at 20 ºC*

3.3.4 Worst case prediction

Using equation 1.6, for triclosan in LDPE (for LDPE, A'_P = 11.5 and τ = 0; for triclosan, $M_{r,\ Triclosan}$ = 289.54), D^*_P equals to 1.50×10^{-07} (60 ºC), 2.02×10^{-08} (40 ºC) and 2.07×10^{-09} (20 ºC). By comparing these results with the ones obtained experimentally (Table 3.2), it is confirmed that D^*_P values are higher than D_E. The same is observed for the D_0 and E_A, where the estimated values (calculated with Eq. 1.4 by using D^*_P values; D_0 = 86.9 $cm^2 \cdot s^{-1}$ and $E_A = 6.36\times10^6$ $kJ \cdot mol^{-1}$) are always higher than D_0 and E_A calculated from the experimental D_E (Table 3.3).

This shows that, accordingly to the results and for any temperature between 20 and 60 ºC, equation 1.6 gives us an overestimated migration.

Table 3.3. *Experimental D_0, E_A and R^2 values calculated with equation 1.5*

Food	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($kJ \cdot mol^{-1}$)	R^2
Gouda cheese	6.8	53.8	0.9390
Turkey ham	6175.2	71.9	1.0000
Cooked ham	1.45×10^{-04}	29.5	0.8705
Pâté	23.4	57.2	0.9868
Tomato sauce	6.87×10^{-06}	22.9	0.9874
Orange juice	2.96×10^{-03}	37.2	0.9765
Red wine	2.22×10^{-04}	30.2	0.9831

However, the curves obtained experimentally will have different slopes than the predicted curve (Fig. 3.4). This happens because the E_A (and consequently, the slope of the curve) predicted with Eq. 1.5 is always 86.9 $kJ \cdot mol^{-1} \cdot K^{-1}$ independently of the migrant, polymers and foodstuff tested. Nevertheless, the experimental E_A will be different if any of these components of the system change. This implies that, at a determined temperature, the experimental curves will cross the "worst case" curve and consequently experimental D_E will be higher than the predicted upper-bound diffusion coefficient, D^*_P. So, when assuming that linearity remains for temperatures lower than 20 ºC, D_E is higher than D^*_P for temperatures between 16.9 and -13.3 ºC accordingly with the foodstuff tested (Gouda cheese: 16.9; pâté: 12.9; red wine: 10.1; cooked ham: 8.7; tomato sauce: 6.1; and orange juice: 5.2; turkey ham: -13.3 ºC. This indicates that in some cases, at real temperature storage conditions (refrigeration temperature = 4 ºC or lower), the

migration of triclosan between LDPE packaging materials and foodstuff might be superior to the predicted. (Fig. 3.4)

3.3.5 Partition Coefficient

Calculated $K_{P/F}$ values are represented in Table 3.2.

Likewise D_E, $K_{P/F}$ depends on several factors, like polarity and solubility of the migrants in the foodstuffs (Tehnary, Mouawad and Desobry, 2007). According to the results, the partition coefficient can be related with the fat content of the selected foods. For the solid foods and pâté (the ones with higher fat content), $K_{P/F}$ is roughly 1, meaning that triclosan is well soluble in the foodstuff, leading to higher migration values; for liquid foods (with low or no fat content), $K_{P/F}$ is at least 15 (lowest value found was 15.9 for orange juice at 20 ºC), indicating that the migrant has higher affinity to the polymer than for these foodstuffs. Nevertheless, turkey ham is the foodstuff where the second highest partition values are found, although pâté and cooked ham have higher fat content. This demonstrates that other factors that not the fat content have significant effects in this parameter.

Unlike D, the partition coefficient is not significantly affected by the temperature (Gandek, Hatton and Reid,, 1989). With the results reported in the present paper, it is possible to see that this happens for the solid foods and pâté (pâté at 20 ºC is the only case were $K_{P/F}$ value stands out, probably because system was not in equilibrium yet). However, for the three liquid foods, where lower migration was observed, greater differences were detected. This does not imply that the storing temperature is responsible for these differences. The reasons for these variations are small errors inherent to the laboratory material and apparatus, migrant differently distributed in the LDPE and/or errors related to the method that may lead to differences in the calculated $K_{P/F}$ values.

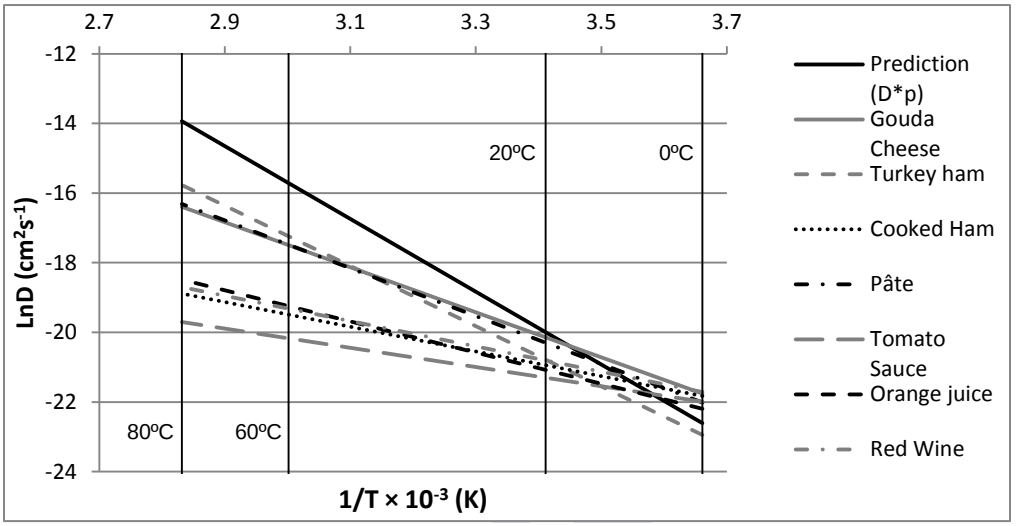

Figure 3.4. *Comparison between predicted D^*_P and experimental D's for temperature ranging between 0 and 80 ºC*

3.4 Conclusions

In the present work, two critical parameters (D_E and $K_{P/F}$) of the migration of triclosan from LDPE into seven different foods were successfully calculated through the application of a simplified mathematical model based on Fick's Second Law of Diffusion. A good correlation was found between experimental and modelled data demonstrating that the applied model is appropriated for estimating migration from polymers into real foodstuffs. Migration modelling is a tool used for checking compliance of FCM with current legislation and, therefore, allows a great reduction of experimental work. These results contribute to a better understanding of the migration process between food and packaging materials.

In this experiment, the factors that have most effect on the migration process were the fat content of the food and the storing temperature. The fat content contributed to a higher migration of triclosan into foodstuff, which was reflected in a higher D_E and lower $K_{P/F}$. Consequently, the higher migration was observed in Gouda cheese and pâté (fat content ≥ 23.0 %), whereas lower migration was found for tomato sauce, orange juice and red wine (fat content ≤ 0.5 %). Nevertheless, other properties linked to the food (physical state, viscosity, alcohol content, pH, fat/water ratio, etc.) may also have some impact in the outcome of the studied mass transfer process.

The storing temperature produced changes of the D_E value (D_E increases with T) but no relation between $K_{P/F}$ and temperature was found. A good Arrhenius correlation was always found between the D_E values for each foodstuff, allowing to accurately estimating the D values for any temperature in the experimental temperature range (between 20 and 60 ºC). The experimental D_E values were compared to the estimated upper-bound D_P values (D^*_P), that represent a worst case scenario. The experimental values never exceeded the predicted ones, at the tested temperature.

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4

Determination of key parameters involved in the migration of 6 model substances from LDPE into pâté and chocolate spread

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Abstract

Packaging is a fundamental practice in the food industry. It is necessary for convenient food transportation, making the product attractive for the consumer and, mainly, to protect the food from spoilage. Unfortunately, these packaging materials used are not inert and may contaminate the foodstuff with some of their components. This phenomenon is known as migration and raises some health concerns because many components from packaging materials are toxic when consumed over some limits.

The aim of this paper is to study the mass transport process (migration) of six selected model substances (benzophenone, 1,4-diphenylbutadiene, Uvitex® OB, diphenylphthalate, 2-isopropylthioxanthone and butylated hydroxitoluene) from LDPE two real foodstuffs (pâté and chocolate spread) at three temperatures (20, 40 and 60 ºC). A mathematical model based on Fick's Second Law of Diffusion was used to simulate the migration process and a good correlation between experimental and predicted values was found. The acquired results will contribute to a better understand of this phenomenon.

The migration tests were made by immersing previously additivated LDPE films in food samples. After a storage period at different time-temperature condition, the LDPE was separated and extracted with ethanol, which was then analysed directly by a HPLC-DAD-FLD method. With the acquired data two key parameters for migration modelling (effective diffusion and partition coefficients) were calculated.

Results showed that the effective diffusion coefficient (D_E) is greatly affected by the temperature and by migrant properties, mainly the molecular weight. The partition coefficient ($K_{P/F}$) was independent of the temperature but greatly depended on the migrant nature. The substance that showed higher migration

values was benzophenone (lower molecular weight) and Uvitex® OB (higher molecular weight) showed the lowest, independently of the temperature.

Keywords: effective diffusion; partition; migration; LDPE; mathematical modelling

4.1 Introduction

Plastics are one of the most used food packaging materials due to some of their advantages over other traditional packaging materials: wide versatility, low weight, flexibility, safety and hygiene, cost advantages, durability, formability into useful and attractive shapes, thermal-sealability, reduced breakage and transparency (Lundquist, Leterrier, Sunderland and Månson, 2000; Finnigan, 2009; Hernandez-Muñoz, Catalá and Gavara, 2001).

Nowadays, the use of polymeric packaging materials is a crucial procedure in the food industry. They provide a physical barrier between the foodstuff and environment, reduce the risk of product contamination, prolong the life of food, provide customers with information and instructions and may even be needed for safe and efficient transportation (Sanches Silva, Cruz Freire and Paseiro Losada, 2010). Nevertheless, because of the nature of the matrix of these materials, some interactions between food and packaging or even environment (through the package) may happen, leading to contamination of the food with undesirable compounds or deterioration of organoleptic characteristics (Canellas, Aznar, Nerín and Mercea, 2010).

When using plastics as food contact materials (FCM), some mass transfer phenomena may occur: permeation, sorption and migration. Depending in the characteristics of the polymer used, the effects can occur in a greater or lesser extent. From these three phenomena, the one that claims more attention from the consumer's safety point of view is migration. Migration is a food-packaging interaction where mainly low weight substances, that are able to move freely in the plastic packaging material and end up being transferred into the foodstuff (Widén, Leufvén and Nielsen, 2004; Sanches Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007; Poças and Hogg, 2007). This phenomenon raises

some concerns regarding human health because some constituents of the FCM (additives, degradation products, residual monomers and oligomers) present some toxicity. Also, because nearly all foods existing in the market are packed/wrapped in packaging materials, FCM are a potential source of food contamination of great importance and interest (Franz, 2005).

Materials and articles intended to come into contact with food (directly or indirectly) are regulated by Regulation No 1935/2004. This regulation is designed to guarantee a correct functioning on the European Union (EU) internal markets of these materials whilst providing the basis for securing a high level of protection of human health and the interests of consumers. Due to plastic importance and widespread within FCM, plastics are also regulated by Commission Regulation (EU) No 10/2011, that includes the rules to check compliance between FCM and legislation, as well as a positive list of all substances allowed to be used in polymer production (monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids). Within this regulation, there are described the allowed overall and specific migration limits. Overall migration limit (OML) means the maximum permitted amount of non-volatile compounds released from a material or article into food simulants (OML = 60 mg [of migrant] $\cdot kg^{-1}$ [of foodstuff]), while specific migration limit (SML) means the maximum permitted amount of a given substance released from a material or article into food or food simulants (Commission Regulation (EU) No 10/2011). OML is not necessarily linked to food safety because it does not exclude the presence of migrants in concentrations high enough to threat to human health or, in contrast, an OML exceeding 60 $mg \cdot kg^{-1}$ does not represent mandatorily a risk (Grob, Pfenninger, Pohl, Laso, Imhof and Rieger, 2007).

The traditional approach to acquire migration data consists on measuring the concentration of migrants directly in the foodstuffs or in authorized food stimulants after being contaminated with an highly additivated FCM. Nevertheless, these

tests requires great amounts of time, money and human resources and are frequently rather difficult from the analytical point of view, due to the complexity of food matrices, especially when using fatty foods or oils (Sanches Silva, Sendón García, Cooper, Franz and Paseiro Losada, 2006). As an alternative to overcome these problems, it is possible to use validated mathematical diffusion models, supported by scientific evidences, to predict the migration process. These advantages make migration modelling a subject of great interest. These models should give an estimated migration close to the real one, but slightly overestimated. This way, there is a safety margin between predicted and real migration. Nonetheless, when non-compliance of FCMs is demonstrated through mathematical model, results are not definitive and must be confirmed by traditional analytical tests (Petersen, Trier and Fabech, 2005).

The mass transfer of plastic packaging components into food, although very complex, is a predictable physical process that generally obeys to Fick's Second Law of Diffusion (Hamdani, Feigenbaum and Vergnaud, 1997; Brandsch, Mercea, Tosa and Piringer, 2002). For migration modelling, two indispensable parameters are needed: the effective diffusion coefficient (D_E) and partition coefficients ($K_{P/F}$). D_E (kinetics) represents the rate at which the model substance migrates from the FCM into the foodstuff, while $K_{P/F}$ (thermodynamic equilibrium) is the ratio of migrant concentration in the FCM and its concentration in the food at equilibrium (Granda-Restrepo *et al*, 2009; Tehrany and Desobry, 2004).

The aim of this work is to determinate the migration parameters D_E and $K_{P/F}$ of six selected model migrants between a previously additivated low density polyethylene film and , in order to better understand this mass transfer process. Furthermore, the acquired data will be used in project FACET (http://www.ucd.ie/facet/aboutfacet/) to develop a mathematical modelling tool that estimates migration between FCM and foodstuffs under real conditions of use, with both deterministic and probabilistic outputs for exposure estimation.

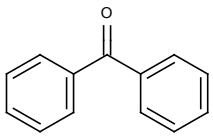
4.2 Experimental

4.2.1 Migrants, chemicals and standard solutions

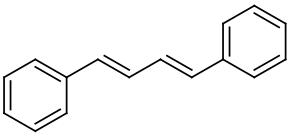
A total of six model migrants selected for this study: benzophenone (BZP; CAS no. 119-61-9), 1,4-diphenylbutadiene (DPBD; CAS no. 538-81-8), Uvitex® OB (CAS no. 7128-64-5), diphenylphthalate (DPP; CAS no. 84-62-8), 2-isopropylthioxanthone (ITX; CAS no. 5495-84-1) and butylated hydroxitoluene (BHT; CAS no. 128-37-0). BZP (purity ≥ 99 %), DPBD (purity 98 %), ITX (purity 97%) were supplied by Sigma Aldrich (Steinheim, Germany), while Uvitex® OB (purity ≥ 99.0 %), BHT (purity ≥ 99.0 %) was supplied by Fluka. Data about physicochemical properties and chemical structure of these migrants are summarized in Fig 4.1.

Ethanol (EtOH; absolute for analysis), acetonitrile (ACN; for liquid chromatography), methanol (MeOH; for liquid chromatography), tetrahydrofuran (THF; for liquid chromatography) were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA, USA). Sodium azide (purity 99%; CAS no. 26628-22-8) was supplied by Sigma Aldrich (Steinheim, Germany).

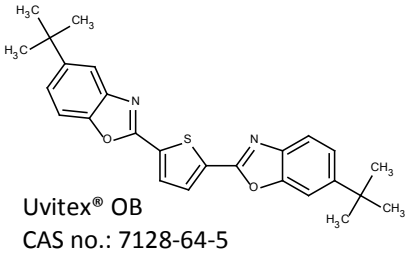
Stock solutions of each migrant were prepared with EtOH with an accurately known concentration of approximately 500 $\mu g \cdot ml^{-1}$. Calibration standard solutions containing the model substances were prepared with EtOH. The concentration of these solutions ranged from 0.05-10 $\mu g \cdot ml^{-1}$. Solutions were stored at 4 ºC and protected from light.



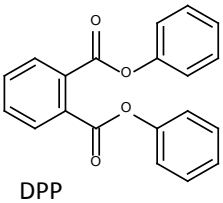
Benzophenone
CAS no.: 119-61-9
MW: 182.2
MP: 47.8 ºC[b]
BP: 305.4 ºC[b]
Log P: 3.18[b]



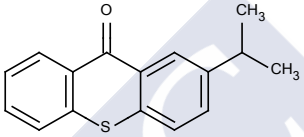
DPBD
CAS no.: 886-65-7
MW: 206.3
MP: 152.0 ºC[b]
BP: 350.0 ºC[b]
Log P: 4.50[a]



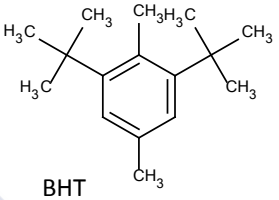
Uvitex® OB
CAS no.: 7128-64-5
MW: 430.6
MP: 198.5 ºC[b]
BP: 531.2ºC[a]
Log P: 7.22[a]



DPP
CAS no.: 84-62-8
MW: 318.3
MP: 73.0 ºC[b]
BP: 255.0 ºC[b]
Log P: 3.567[a]



ITX
CAS no.: 5495-84-1
MW: 254.4
MP: 77.5 ºC[b]
BP: 398.9 ºC[a]
Log P: 5.113[a]



BHT
CAS no.:128-37-0
MW: 220.4
MP: 71.0 ºC[b]
BP: 265.0 ºC[b]
Log P: 5.36[b]

Figure 4.1. *Selected migrants chemical structure and physicochemical characteristics (Data obtained from Scifinder® database; a – predicted; b – experimental)*

4.2.2 Plastic film

In this work low density polyethylene films (LDPE) was selected as a migrant source. This polymer was selected because it is one of the most widely used polymers in food packaging and because it has one of the highest diffusion rates among polymers, contributing to give a safe margin, an very important aspect in food safety studies (Brandsch, Mercea and Piringer, 2000; Cruz, Sanches Silva,

Sendón García, Franz and Paseiro Losada, 2007). In order to simplify the analysis, the model substances were separated into two groups when added into the LDPE film: (1) BZP, DPBD and Uvitex® OB; (2) DPP, ITX and BHT.

The film with BZP, DPBD, Uvitex® OB (457.8±14.1 µm thick) was produced by Fraunhofer IVV (Freising, Germany) and additivated with BZP, DPBD and Uvitex® OB, performed through an immersion process. Sheets of the polymer were immersed in a highly concentrated ethanolic solution containing the three model migrants (5000 $mg \cdot L^{-1}$ of BZP; 2000 $mg \cdot L^{-1}$ of DPBD; 2000 $mg \cdot L^{-1}$ of Uvitex® OB). The container was hermetically closed and stored for 48 hours at 60 ºC. At the end of this period, the sheets were washed twice by quick immersion in fresh EtOH and dried on a kitchen paper towel for about 30 minutes. The concentration achieved in the LDPE was 412.4±41.8 $\mu g \cdot g^{-1}$ for BZP, 721.5±9732 $\mu g \cdot g^{-1}$ for DPBD and 806.1±15.9 µg·g-1 for Uvitex® OB.

The film additivated with DPP, ITX and BHT was produced by GAIKER Technology Centre (Zamudio, Spain). This film thickness was 398.1±10.0 nm. The additivation of the plastic was done during production of the film by an extrusion process. The initial concentrations of DPP, ITX and BHT in the LDPE film were 358.9±56.8 $\mu g \cdot g^{-1}$, 1107.3±178.6 $\mu g \cdot g^{-1}$, 821.2±129.0 $\mu g \cdot g^{-1}$, respectively.

4.2.3 Foodstuff

For this work, pâté and chocolate spread were selected as test foodstuffs and they were kindly supplied by Nestlé. Both are semi-solid food with high viscosity, high fat content (23.0 and 31.0 %, respectively) but they greatly differ in water content. While pâté has high water content (61.1 %, resulting in a fat/water ratio of 0.38), chocolate spread has a very low water content (<2 %, resulting in a fat/water ratio higher than 15.5).

4.2.4 Migration test

For this work, additivated LDPE samples were placed in contact with selected foodstuff under controlled time-temperature conditions.

Film samples previously additvated were cut in 10 cm^2 pieces and placed in contact with 20.0 g of foodstuff accurately weighted in a 30 ml amber flask. A small quantity of sodium azide was added to prevent food spoilage due to microbial growth. The flask were tightly closed and placed in an oven. Three temperatures were chosen: 20, 40 and 60 ºC. The contact times between LDPE and foodstuff for all temperatures are specified in the Table 4.1. Samples were prepared always in duplicates.

Table 4.1. *Migration test conditions*

Temperature (ºC)	Time (h)
60	0.5; 1; 2; 4; 8; 12; 24; 48; 72; 168
40	1; 2; 4; 8; 12; 24; 48; 72; 168; 240
20	2; 4; 8; 12; 24; 48; 72; 168; 240; 360

4.2.5 Sample preparation

The determination of a migrant transfer into a food simulant or food system is usually a difficult complex task due to analytical problems related with the complexity of food matrices, non-availability of an appropriate analytical method and other problems already stated in this work (O'Brie, Cooper and Tice., 1997). To avoid these problems, instead of using the traditional approach, i.e.

determining the migrant concentration directly from the foodstuff, the migrant was determined from the LDPE film. The concentration of the model substances in the food was indirectly calculated as the difference between the initial and sample concentrations in the polymer.

At the end of each storage time (Table 4.1), a sample was taken from the laboratory oven. The LDPE film was separated from the foodstuff and, after being carefully cleaned with a paper tissue, the polymer was extracted with 50 ml of EtOH at 60 ºC for 6 hours. After this time, the flask was taken out of the oven, an EtOH aliquot was taken, filtered with a 0.45 um filter and analysed directly by HPLC.

In order to verify if the extraction had been complete, the plastic film was cleaned with EtOH to remove any remaining migrant that came from the ethanolic extraction solution and it was submitted to a second extraction under the same conditions.

4.2.6 Chomatographic condition

High performance liquid chromatography with ultraviolet and fluorescence detection was performed using a Hewlett-Packard HP 1200 chromatograph equipped with a diode array detector (DAD) and a fluorescence scanning detector (FLD) arranged in serie. The conditions used for determination of the model substances are listed in Table 4.2.

Table 4.2. *HPLC conditions*

	Film 1	**Film 2**
Apparatus	The HPLC system model 1200 HP (Hewlett-Packard, Waldbronn, Germany)	Same
Column	Kromasil C18 25×0.36 I.D., 5 µm particle size	Same
Injection volume (µl)	20	10
Column temp. (ºC)	30	30
Detection (nm)	DAD: 256 (BZP) 330 (DPBD) 372 (Uvitex® OB)	DAD: 205 (DPP and BHT); FLD: Ex. 250; Em. 410 (ITX)
Mobile phase	A: Milli-Q water B: THF 30 % in MeOH	A: Milli-Q water C: ACN
Flow	0.5 ml·min^{-1}	0.5 ml·min^{-1}
Gradient	0.0 min 30.0 % A 70.0 % B 4.0 min 30.0 % A 70.0 % B 10.0 min 0.0 % A 100.0 % B 17.0 min 0.0 % A 100.0 % B	0.0 min 40.0 % A; 60.0 % C 1.0 min 40.0 % A; 60.0 % C 17.0 min 0.0 % A; 100.0 % C 20.0 min 0.0 % A; 100.0 % C
Software	HP ChemStation	HP ChemStation
Retention time (min)	BZP: 5.3 DPBD: 12.7 Uvitex® OB: 14.9	DPP: 11.7 ITX: 16.8 BHT: 17.8

4.3 Results and discussion

4.3.1 Migration parameters: D_E and $K_{P/F}$

Migration parameters $K_{P/F}$ (the ratio between the migrant concentration in the polymer and food at steady state, i.e. the relative solution of the migrant between the plastic and the foodstuff at equilibrium) and D_E (the parameter that describes the kinetics of the migration process in a FCM/food system) were successfully determined by fitting the experimental data in the proposed mathematical models (Eq. 1.2 and 1.3) and RMSE values were (Eq. 1.8) was calculated. These values are shown in Table.4.3.

To accurately determinate $K_{P/F}$, similar migrant concentrations should be found in both film and foodstuff, because when migration is very low (high $K_{P/F}$) or very high (low $K_{P/F}$) errors inherent to the method, heterogenic food matrix, polymer thickness variations or migrant not homogeneously distributed in the film become more significant and, consequently, some differences between calculated and real $K_{P/F}$ may exist. With fatty foodstuffs, such as ones used in this work, great amounts of substances usually migrate into the food, reflected by a low $K_{P/F}$ in the results. Also, it is important to reach the equilibrium. Most of the samples reach the equilibrium, but for Uvitex® OB (at all temperatures), BHT and DPBD (both at 20ºC only) in pâté and BHT, DPP and Uvitex® OB (at 20ºC) in chocolate spread assays equilibrium was not attained. For these two reasons, the results should be interpreted with caution.

The differences observed in the $K_{P/F}$ values between both foodstuffs show that the migrants used (which have a lipophilic tendency) have more affinity for the chocolate spread than for the pâté, although their differences in fat content it is not very significative (31 and 27% respectively). This indicates that there is

Table 4.3. *D_E, $K_{P/F}$ and RMSE values for the migration of the model substances into pâté and chocolate spread*

Migrant	Temp. (ºC)	Pâté			Chocolate Spread		
		D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$	RMSE (%)	D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$	RMSE (%)
BZP	60	3.27×10^{-8}	2.64	3.5	6.93×10^{-8}	0.80	0.98
	40	1.53×10^{-8}	1.70	1.6	2.57×10^{-8}	0.40	1.54
	20	4.11×10^{-9}	3.11	4.0	4.24×10^{-9}	0.68	0.54
DPBD	60	6.22×10^{-9}	14.17	6.7	4.29×10^{-8}	0.80	1.31
	40	1.67×10^{-9}	4.77	4.9	1.43×10^{-8}	0.51	2.05
	20	3.83×10^{-10}	12.19	3.2	1.69×10^{-9}	1.37	1.04
Uvitex® OB	60	1.81×10^{-9}	17.64	5.9	5.31×10^{-9}	0.056	5.35
	40	4.41×10^{-10}	5.53	3.2	1.40×10^{-9}	1.16	6.17
	20	4.74×10^{-11}	6.65	0.94	6.83×10^{-11}	6.89	3.27
DPP	60	1.76×10^{-8}	2.35	2.6	4.75×10^{-8}	1.98	3.07
	40	4.23×10^{-9}	1.79	1.5	4.45×10^{-9}	1.77	2.91
	20	6.67×10^{-10}	3.75	2.8	4.63×10^{-10}	1.75	2.13
ITX	60	7.34×10^{-9}	6.96	7.7	3.98×10^{-8}	0.92	1.02
	40	2.69×10^{-9}	7.91	4.9	8.70×10^{-9}	0.70	1.58
	20	7.65×10^{-10}	12.03	4.0	9.74×10^{-10}	0.80	1.15
BHT	60	6.41×10^{-9}	13.20	6.0	2.78×10^{-8}	0.28	1.95
	40	1.65×10^{-9}	15.42	4.2	4.86×10^{-9}	0.17	2.93
	20	3.00×10^{-10}	26.39	3.7	6.78×10^{-10}	3.61	5.59

another factor that greatly affects the migrant equilibrium in the LDPE/food system. This factor seems to be the fat/water ratio. In the case of pâté, this ratio is 0.38, while in chocolate spread, which has much less water in its composition, it is 15.5.

As such, migrants with lipophilic properties will have a higher tendency to migrate more extensively into the foodstuff, resulting in lower $K_{P/F}$.

By looking to D_E values displayed in Table 4.3, it is clear that D_E increases with the temperature. The effect of storage temperature can also be observed the example shown in Fig. 4.2. D_E linearity between the experimental D_E values obtained at the three experiment temperatures was checked with Eq. 1.5.

The D_E values have shown a good linearity ($R^2 \geq 0.9668$), demonstrating that it is possible to estimate the D_E value for any temperature between 20 and 60 ºC. The E_A and D_0 values were also determined (Table 4.4).

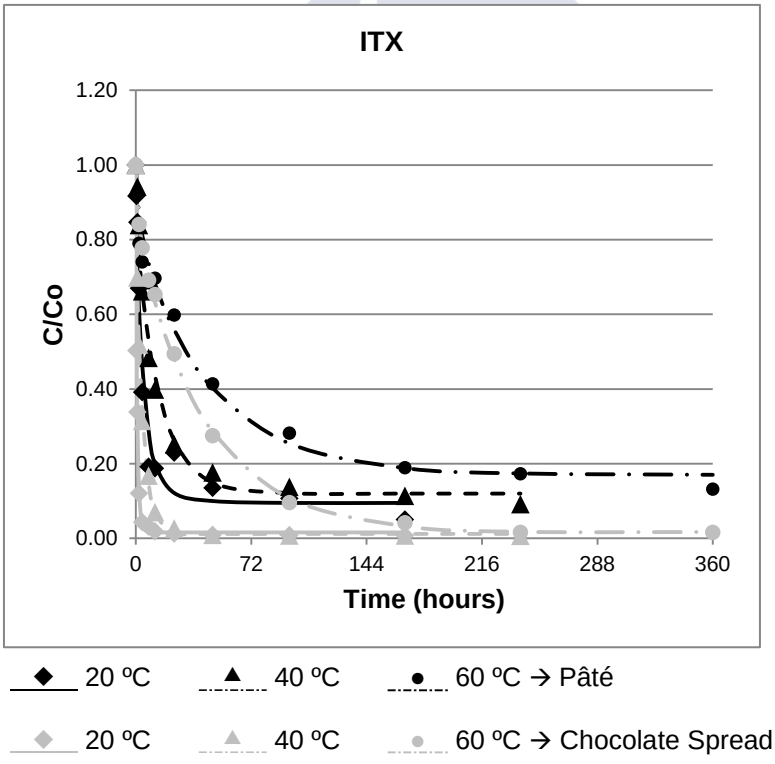


Figure 4.2. *Migration of ITX from LDPE into pâté and effect of temperature*

The physicochemical properties of the migrants also play a crucial role in the diffusion coefficient. Many papers indicate MW as the most important factor (higher MW contributes to a lower diffusion), but others characteristics may also have a significant effect. Uvitex® OB (the tested migrant with higher MW) has the lowest D_E, independently of the temperature; while BZP, by having the lowest MW, has the highest D_E for the three temperatures. For the other substances this correlation between MW and D_E is not observed and D_E values are generally very similar. This may be the result of other characteristics that have enough influence to surpass effects caused by the small differences in the MW of the migrants. Polarity, molecular shape, volatility, chemical functional groups, solubility of the migrant to the foodstuff and polymer are some of these physicochemical properties more commonly described in the bibliography (Helmroth, Dekker and Hankemeier, 2002; Feigenbaum *et al*, 2005; Hamdani, Feigenbaum, 1996). An example comparing the migration behaviour of the different migrants is shown in Fig. 4.3. For DPP, these characteristics appear to have a very important role because, from the tested substances, this is the one with the second highest MW (MW = 318.3) and, even though, is the one that has the second highest D_E at 60 ºC and, at 40 and 20 ºC, its D_E value is comparable (some times even higher) to the D_E of DPBD, ITX and BHT, that have a much lower MW.

Other important factor, this time related with the foodstuff, that affects D_E is the fat content. Accordingly to results shown in table 4.3, D_E is always higher when chocolate spread is used (except for DPP at 20ºC). This was expected, because this foodstuff has a higher fat content than pâté. Nevertheless, the fat/water ratio might also have a role that influences the D_E by increasing the kinetics of the migration. A comparition between the migration behaviour in both foodstuffs in shown in Fig 4.2 for the case of ITX.

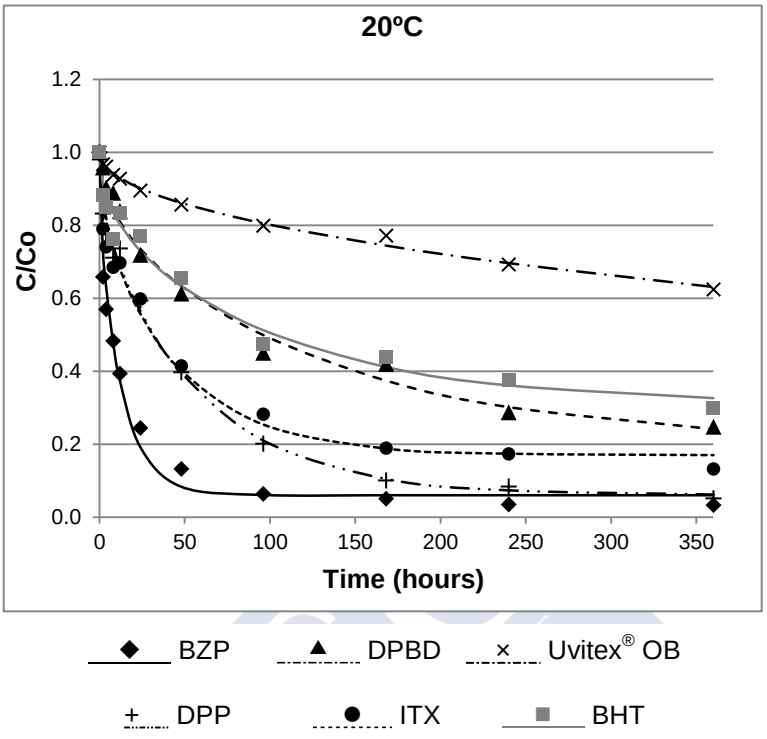


Figure 4.3. *Migration of model migrants from LDPE into pâté*

As it was cited previously in Chapter 1, an upper-bound diffusion coefficient within a specific polymer (D^*_P) can be predicted with Eq. 1.6. D^*_P was calculated for all model substances and temperatures used. When compared with the experimental D_E, we verify that, without exception, D^*_P is always higher. Nonetheless, when calculating D_0 and E_A for D^*_P values (Table 4.4) and assuming that linearity would remain for temperatures below 20 ºC, it is possible to observe that when the temperature decreases D^*_P and D_E values get closer until, at some point, D^*_P is surpassed by D_E and no longer represents a worst case scenario. The temperatures were $D^*_P \geq D$ are shown in Table 4.4. For DPBD, Uvitex® OB and BHT this happens for extremely low temperatures (lower than -11.0 ºC), where is not likely to have food stored at that conditions. On other hand, for the rest of the

model migrants, this happens at more realistic storage conditions (between 12.3 and -5.6 ºC). Amongst these results, there are two cases that present a different behaviour than the rest, the DPP and Uvitex® OB, both in chocolate spread. In the first case, D_E will theoretically surpass D^*_P at temperatures above 254.7ºC, not likely the other cases, where the condition $D_E > D^*_P$ is more likely to happens and more intense along decreasing temperatures. In the second case, D_E will always be higher than D^*_P for any temperature.

Table 4.4. *Pre-exponential factor and activation energy; Comparison between experimental and predicted values*

Food	Migrant	Experimental			Estimation		Min. T (ºC) for $D_E > D^*_P$ [a]
		D_0 ($cm^2 \cdot s^{-1}$)	E_A ($kJ \cdot mol^{-1}$)	R^2	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($kJ \cdot mol^{-1}$)	
Paté	BZP	0.15	42.3	0.9862	2.22×10^7	86.9	12.3
	DPBD	4.6	56.6	1.0000	1.64×10^7	86.9	-31.2
	Uvitex® OB	867.2	74.2	0.9915	1.62×10^6	86.9	-70.2
	DPP	489.6	66.5	0.9986	4.72×10^6	86.9	-5.6
	ITX	0.12	46.0	0.9993	9.34×10^6	86.9	-2.0
	BHT	37.7	62.2	0.9992	1.38×10^7	86.9	-41.4
Chocolate Spread	BZP	66.5	57.0	0.9834	2.22×10^7	86.9	10.0
	DPBD	1086.2	65.9	0.9786	1.64×10^7	86.9	-11.0
	Uvitex® OB	6.09×10^5	89.0	0.9668	1.62×10^6	86.9	-
	DPP	2.28×10^7	93.8	0.9975	4.72×10^6	86.9	-[b]
	ITX	2.97×10^4	75.5	0.9955	9.34×10^6	86.9	-34.6
	BHT	1.83×10^4	75.4	1.0000	1.38×10^7	86.9	-63.8

[a] assuming that linearity would remain out of the experimental temperatures range

[b] while in all cases, below a certain T, experimental D_E will always surpass D^*_P (theoretically), in the case DPP in chocolate spread, D_E will always surpass D^*_P only when T is higher than 254.7

4.4 Conclusions

The number of studies on the migration of substances into real foodstuffs is still quite scarce. Consequently, the results attained in this work may contribute to a better understand of this mass transfer process.

This study provides results that give consistent data about the migration of six model migrants from LDPE into pâté, a semi-solid foodstuff with high consistency and high fat and water content, and chocolate spread, also a a semi-solid foodstuff with high consistency and high fat content, but with very low water content. Two essential parameters for migration modelling (D_E and $K_{P/F}$) were successfully calculated and a good linearity between D_E values was achieved, allowing estimating D_E values for any temperature between the experimental temperatures conditions that were used.

The migration process was simulated through a mathematical model based on Fick's Second Law of Diffusion. A good correlation between experimental and simulated values was obtained, indicating that this model is appropriated for migration modelling and, therefore, may be useful for check compliance with the current legislation. Migration modelling has several advantages when compared with laboratory tests, because it allows reducing costs, time and human resources.

Molecular weight of the migrant greatly affected the diffusion coefficient, although it is not the only migrant properties involved in D_E. Others as polarity, affinity to the foodstuff and volatility also play an important role in the kinetic behaviour of migration.

Regarding foodstuff composition, the high fat content is regarded has the most important factor, but our results indicate that the fat/water ratio may produce different migration behaviour in foddstuffs with similar fat content.

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5

Diffusion of model substances from LDPE films into real foodstuffs

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Abstract

The transfer of substances from food packaging into foodstuff is known as migration. This phenomenom may represent a risk to the consumer health due the possible toxic effects of the migrating substances.

Migration is an inevitable phenomenom. Therefore, in order to protect consumers, it is necessary to understand it in order to ensure that the consumers are not ingesting chemical substances in amounts that may be harmfull for their health.

This mass transfer process between packaging and food is a complex process and it will depend on several factors, namely, the physic-chemical properties of the migrants (being the molecular weight the most important), type of packaging material and foodstuffs, direction of the contact between food and packaging and temperature. Still, it is predicable and can be estimated mathematically. The aim of this work is to determine two migration modeling key parameters in a system composed by a LDPE film and foodstuff. These parameters are the partition and effective diffusion coefficients ($K_{P/F}$ and D_E). In this experiment, the model migrants used were BZP, DPBD and Uvitex® OB, and the foodstuffs used were wine, tomato sauce and cooked ham.

Migration kinetics were prepared by placing a LDPE additivated with different foodstuffs at controlled time-temperature conditions. Afterward, the remaing migrant in the plastic was extracted and quantified by HPLC-DAD. By calculating the difference between the final and initial quantity of migrant in the polymer, it was possible to calculate the their concentration in the foodstuff.

The key parameters $K_{P/F}$ and D_E were calculated by fitting the experimental data with a model based in Fick's Second Law of Diffusion. A good fitting between

experimental and the proposed model was found, and the migration parameters were sucessufully calculated.

The results shown that the migration is faster (higher D_E) and more extensive (lower $K_{P/F}$) for migrants with low molecular weigh and with cooked ham (only foodstuff with a significative fat content). D_E was also affected by the storage temperature, but no relation between temperature and $K_{P/F}$ was found.

Keywords: migration modeling; effective diffusion coefficient; partition coefficient; LDPE;

5.1 Introduction

Over the last decades, interactions between food contact materials (FCM) and foodstuffs have been widely studied. Yet, these studies require great amounts of time and are costly, even when food simulants are used instead of real foodstuff. Also, it is impossible to test all of possible combinations between food, FCM and potentially migrating substances through usual experimentation because there is as endless list of them and/or due to analytical/technical problems related with the complex matrix that foods are. For these reasons, it is necessary a different way to better execute this task. (Brandsch, Mercea, Rüter, Tosa and Piringer, 2002; Begley *et al*, 2005)

Migration (the mass transfer of mainly low molecular weight substances, such as, additives, degradation products, impurities, residual monomers and oligomers and/or other starting substances, that are able to move freely from FCM into foodstuff) from polymeric materials is a complex but predictable physical process that, most of the times, can be described through Fick's Second Law of Diffusion (Feigenbaum *et al*, 2002; Brandsch, Mercea, Rüter, Tosa and Piringer, 2002). Even though migration is not the only interaction between FCM and foodstuff, it is the most important phenomenon from the health safety standpoint due to possible hazardous effects of some of the constituents of FCM that might migrate into foodstuff (Sanches Silva, Cruz Freire, Franz and Paseiro Losada, 2008).

To control the safety of polymeric FCM in Europe, it has been established in the EU Commission Regulation no. 10/2011 a positive list of substances that are allowed in the production of polymers and respective specific migration limits (SML), that is "the maximum permitted amount of a given substance released from a material or article into food or food simulants". In the same regulation it has also

been established an overall migration limit (OML), defined as the "maximum permitted amount of non-volatile substances released from a material or article into food simulants". This OML restriction is not related with food safety, because it is too high (60 $mg \cdot kg^{-1}$) to guarantee the inexistence of migrants in concentrations high enough to represent a risk to human health; or, when this limit is surpassed, does not mean that there is an actual threat. In the current legislation, there is also a set of strict rules and conditions required to perform migration studies but, as it was said previously, this is a difficult or sometimes even impossible task.

One approach to overcome these issues consists in the use of validated migration models. Limm and Hollifield (1996), Piringer (1994) and many other authors have already discussed these models. These are intended to slightly overestimate the migration so that there is a safety margin. Furthermore, it is interesting to calculate maximum initial concentration (MIC) of substance allowed in a polymer so that the migration does not exceed the limits stated in the EU Commission Regulation no. 10/2011. The estimation of migration by mathematical modelling has already being introduced in the European Union (EU) in 2002 as a tool for verification of compliance with the legislation. Unfortunately, sometimes these mathematical models do not work correctly, because real foodstuffs are very complex matrices and information about it is sometimes insufficient (Sanches Silva, Cruz, Sendón García, Franz and Paseiro Losada, 2007a). Even so they are a very useful tool with great potential and, when non-compliance with legislation is shown, the prediction must be confirmed by verification method.

The present work, framed within the FACET project (Flavourings, Additives and food Contact materials Exposure Task), was undertaken to study the migration process of three model substances (benzophenone, diphenylbutadiene and Uvitex® OB) from low density polyethylene (LDPE) into three foodstuffs (red wine, tomato sauce and cooked ham) at three different temperatures (20, 40 and 60 ºC). With the acquired data, two key parameters for migration modelling were

calculated: the diffusion coefficient (D) and the partition coefficient ($K_{P/F}$). These are the parameters that describe behaviour of a substance being transferred from packaging materials into foodstuffs. D represents the rate of transfer of the migrant from the FCM into the food (effective diffusion coefficient - D_E), i.e. the kinetic of the process; $K_{P/F}$ is the ratio of the migrant concentration between the polymer and foodstuff at steady state, which is the thermodynamic equilibrium of the phenomenon (Ossberger, 2009).

The achieved data is meant to be used in project FACET that has the purpose of develop a mathematical modelling tool based on Fick's Second Law of Diffusion to predict migration from food packaging materials into real foods under realistic conditions and to develop validated software for deterministic and probabilistic exposure modelling of food chemical intake.

5.2 Materials and methods

5.2.1 Food samples

For this study, a total of three different foodstuffs with very different characteristics and composition were chosen. Each one of them represents a general group of foods: cooked ham – solid food with rich in protein and fat; tomato sauce – liquid food with solid particles in suspension and very low protein and fat content; Red wine – aqueous liquid food with ethanol content and no fat in its composition. All these products are highly consumed in Europe. These foods composition is detailed in Table 5.1.

Cooked ham and red wine samples were purchased in a local supermarket, while tomato sauce samples were kindly supplied by Nestlé.

Table 5.1 *Foodstuff composition*

Food	General description	Fat %	Protein %	CH %	Water %	Fat/Water ratio
Tomato sauce	Liquid food with solids in suspension and very low protein and fat content.	1.3	0.20	8.7	89.1	0.015
Red wine	Aqueous liquid food with ethanolic content. No fat content.	0.00	0.07	2.6	86.5	0.00
Cooked ham	Solid food with high water and protein contents. Medium fat content.	7.6	18.8	0.69	71.8	0.11

data acquired from USDA database; CH - carbohydrates

5.2.2 Chemicals and standard solutions.

Ethanol (EtOH; absolute for analysis), acetonitrile (ACN; for liquid chromatography) were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA, USA). Sodium azide (purity 99%; CAS no. 26628-22-8) was supplied by Sigma Aldrich (Steinheim, Germany).

For this work, a three model migrants with different physicochemical characteristics were tested: benzophenone (BZP; CAS no. 119-61-9), 1,4-diphenylbutadiene (DPBD; CAS no. 886-65-7) and Uvitex® OB (CAS no. 7128-64-5). BZP can be used as a photo initiator in UV-curing applications and is a good

wetting agent for pigments (Anderson and Castle, 2003). It has a molecular weight (MW) of 182.2 g·mol^{-1}, a melting point (MP) of 47.8 ºC, a boiling point (BP) of 305.4 ºC and a log P (o/w) of 3.18. DPBD is a fluorescent whitening agent that absorbs UV light and release them as blue rays, giving the impression that the plastic is whiter (Sanches Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007b). Its physicochemical properties are: MW = 206.3 g·mol^{-1}; MP = 152 ºC; BP = 350 ºC; log P (o/w) = 4.50. For last, Uvitex® OB, that is also a fluorescent whitening agent, an ultraviolet (UV) stabilizer and an effective heat stabilizer (Sanches Silva, Sendón García, Cooper, Franz, Paseiro Losada, 2006), it has a MW of 430.6 g·mol^{-1}, MP of 198.5 ºC, BP of 531.2 ºC and log P (o/w) of 7.22. All the physicochemical properties were obtained from SciFinder® database. BZP (purity ≥ 99 %) and DPBD (purity 98 %) were supplied by Sigma Aldrich (Steinheim, Germany), while Uvitex® OB (purity ≥ 99 %) was supplied by Fluka.

Three stock solutions (one of each migrant), with an accurately known concentration of approximately 500 µg·ml^{-1}, were prepared in EtOH. Calibration standard solutions containing the three model substances, with concentrations ranging from 0.05 to 10 µg·ml^{-1}, were prepared by diluting the sock solutions with EtOH. Solutions were stored at 4 ºC and protected from light.

5.2.3 LDPE samples

LPDE films were selected as migrant reservoir. This polyolefin was selected for two main reasons: (1) it is widely used as food packaging material; (2) polyolefins in general, and particularly LDPE, have the highest diffusions coefficients amongst the polymers. As such, the use of LDPE grants a worst case scenario overview (Cruz, Sanches Silva, Sendón García, Franz and Paseiro Losada, 2008).

The LDPE polymer has produced by Gaiker and the three migrants were added during the production of the film by an extrusion process. The film has 415.4±25.4 µm and 0.92 g cm^{-3} density. The concentration of the model substances were 510.8±63.2 $mg \cdot kg^{-1}$ for BZP, 771.8±76.5 $mg \cdot kg^{-1}$ for DPBD and 851.9±63.6 $mg \cdot kg^{-1}$ for Uvitex® OB.

5.2.4 Foodstuff/polymer contact

LDPE film samples measuring 10 cm^2 were cut, precisely weighted and placed in contact with the selected foodstuffs. Depending on the food characteristics, the procedure to allow contact between food and LDPE previously additivated with the three model migrants was slightly different. So, for solid food (cooked ham), two slices were cut with the same dimensions as the LDPE samples and weighted (approx. 3.3 g). Food samples and polymer were then placed together so that the film was in contact with the cooked ham by both sides and the system was slightly pressed to remove all air between food and plastic, ensuring intimate contact between the components of the system. A small quantity of sodium azide (approx. 1 mg) was added to the each face of the cooked ham that was not in contact with the LDPE and the system food/LDPE/food was wrapped in aluminium foil. The sample was then sealed in a polyamine/polyethylene bag to prevent water loss and stored in an oven. For liquid foods (red wine and tomato sauce), the LDPE was placed in a 60 ml flask previously filled with 50 ml of red wine or 50 g of tomato sauce. The flask was tightly closed and place in an oven.

The time-temperatures storage conditions are described in Table 5.2. A total of ten samples were prepared for each food-temperature combination and duplicates were always prepared.

Table 5.2. *Storage time and temperature conditions*

Temperature	Time
20 ºC	2; 4; 8; 12; 24; 48; 72; 96; 168; 240; 360 h
40 ºC	1; 2; 4; 8; 12; 24; 48; 72; 96; 168; 240 h
60 ºC	0.5; 1; 2; 4; 8; 12; 24; 48; 72; 96; 168 h

5.2.5 Migrant extraction and quantification

When performing migration studies, the quantity of migrant that was transferred from the migrant source into the foodstuff (or food simulant) is determined from the foodstuff after an extraction procedure. This is very laborious and it has several inconvenient, specially due the complexity of food matrixes: each food requires a different or adapted method of extraction; sometimes there are any methods available; several interferences may exist; extractions are made by several steps, leading inevitably to losses; recoveries may be very low. To avoid these problems, a different path was taken. Instead of extracting the model substances from the food, they were extracted from the LDPE polymer and the quantity of migrant transferred into the food was calculated by the difference between the initial and final concentrations in the plastic.

A simple extraction method was applied. At the end of each storage period, the additivated films in contact with the foodstuff was separated, cleaned carefully with kitchen paper tissue and placed in a flask filled with 50 ml of EtOH. After being tightly closed, the flask was stored in an oven at 70 ºC for 6 hours. The flask was taken from the oven and after cooling an EtOH aliquot was filtered and analyzed by HPLC-DAD.

After this extraction, migrant residues on the polymer surface were removed by quick immersion in fresh EtOH. A second extraction was performed in the films by submitting the polymer to the same conditions as in the first extraction.

5.2.6 Chromatographic conditions

The HPLC system model 1200 HP (Agilent, Waldbronn, Germany) was fitted with a quaternary pump, a degassing device, an autosampler, a column thermostat system and a diode array detector (DAD). The DAD was continuously performing a scan in the range of 190 to 400 nm.

Chromatographic separation was performed with a Kromasil C18 column (250 × 3.20 mm internal diameter, 5 µm particle size) (Phenomenex, Barcelona, Spain) at 30 ºC. BZP was detected at 256 nm, DPBD was detected at 330 nm and Uvitex® OB was detected at 372 nm.

The mobile phase used in this analysis was composed of a mixture of milli-Q water and a methanolic solution of 30 % THF. During the first 4 minutes, the mobile phase was composed of a combination of 30 % milli-Q and 70 % THF methanolic solution. After this period of time, the THF methanolic solution was increased gradually until reaching 100 % at 10 min. This percentage was maintained until the end of the analysis, at 17 min. The mobile phase flow rate was 0.5 ml·min^{-1} during the entire time of analysis and the sample injection volume was 20 µl. For data acquisition, HP ChemStation® chromatographic software was used. The model migrants were identified by comparison of their retention times and UV spectrum with those of injected standards using the same HPLC conditions.

5.3 Results

5.3.1 HPLC method validation

The HPLC method was calibrated by using series of standard ethanolic solutions containing BZP, DPBD and Uvitex® OB. A set of solutions, with well known concentrations ranging from 0.05 to 10 $mg \cdot L^{-1}$, were analyzed by triplicate. The calibration lines of concentration versus area of chromatographic peaks (average of the three measurements) were attained by linear regression. Ten samples of 1.0 $ug \cdot ml^{-1}$ were analyzed and relative standard deviations (RSD%) were calculated, ranging from 0.37 to 0.48 % for run to run precision.

Good linearity was obtained for the three model substances ($R2 \geq 0.9997$), meaning that the method was appropriated for quantification. Slope and intersect parameters are 217.3 and -1.607 for BZP, respectively; 587.0 and 13.81 for DPBD, respectively; and 266.6 and 8.944 for Uvitex® OB, respectively.

5.3.2 Migration key parameters

The migration key parameters D_E and $K_{P/F}$ were successfully calculated after fitting the experimental data with the proposed model (Eq. 1.2 and 1.3) by non-lineal regression. Their values are shown in Table 5.3.

RMSE values were always low ($RMSE \leq 5.1$ %), meaning that a good correlation between experimental and estimated data was achieved and that the proposed model is valid for predicting the migration transfer of model substances from FCM into foodstuffs.

Effective diffusion coefficient

As it was said previously, one of the key parameter that allows predicting the migration process is D_E. This variable is related with the kinetics of the migrant in the process, i.e. the rate at which the model substance migrates from the packaging into the food (Ossberger, 2009), and it depends on several factor as migrant, food characteristics and storage conditions.

By observing the obtained D_E values, it is clearly seen that there is a correlation between D_E and temperature. When the temperature increases D_E also increases, meaning that the transfer between LDPE film and food is faster at higher temperatures.

To check linearity between D_E and temperature, the Arrhenius type equation 1.5 was used. With this eqution, D_0, E_A and R^2 values were calculated (Table 5.3). Good correlations coefficients were found, confirming that with Eq. 1.5 allows estimating the D_E values for any temperature between 20 and 60 ºC.

D_E also depends on the kind of migrant used. Many physicochemical characteristics intervene in the migration process, but the one that is greatly pointed by many authors is the MW of the migrant. This is due the fact that it the migration rate considerably increases when the migrating substance MW decreases (< MW = > D) and also because is a parameter that can be easily determined. BZP, the lowest MW substance used in this study, shows much higher migration rates than DPBD and Uvitex® OB for the three model foodstuffs. Nevertheless, between DPBD (slightly higher MW than BZP MW) and Uvitex® OB (much higher MW than the other two model substances), no major differences are found. In fact, with tomato sauce, D_E values for Uvitex® OB are higher than for DPBD. This may be justified due to the interference of other parameters or due to errors inherent to calculation that tend to loss of accuracy when very little quantities of migrant (or most migrant present in the polymer) migrate into food.

Table 5.3. *D_E, $K_{P/F}$ and RMSE values for model migrants in contact with selected foodstuffs at different temperatures*

Food	Temp. (ºC)	BZP			DPBD			Uvitex® OB		
		D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$	RMSE (%)	D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$	RMSE (%)	D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$	RMSE (%)
Tomato sauce	20	6.9×10^{-10}	35.8	1.9	9.8×10^{-12}	>1000	5.1	2.6×10^{-11}	>1000	1.3
	40	1.4×10^{-09}	41.5	1.7	2.2×10^{-11}	>1000	2.8	1.7×10^{-10}	>1000	0.96
	60	3.2×10^{-09}	33.6	3.2	3.4×10^{-11}	>1000	3.7	1.3×10^{-09}	>1000	1.5
Red Wine	20	1.9×10^{-09}	53.6	1.1	nm	nm	-	nm	nm	-
	40	6.5×10^{-09}	42.3	3.0	nm	nm	-	nm	nm	-
	60	1.3×10^{-08}	32.2	2.1	nm	nm	-	nm	nm	-
Cooked ham	20	3.6×10^{-09}	2.6	4.1	5.4×10^{-12}	49.5	1.0	2.2×10^{-12}	90.0	1.8
	40	6.2×10^{-09}	4.0	2.6	1.7×10^{-11}	28.5	1.4	3.1×10^{-12}	52.2	2.0
	60	9.9×10^{-09}	6.1	2.4	3.7×10^{-11}	26.6	2.2	1.5×10^{-11}	271.1	1.3

Table 5.4. *Activation energy and pre-exponential of the migration process*

Migrant	Tomato sauce			Red Wine			Cooked Ham			Theoretical prediction	
	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($J \cdot mol^{-1}$)	R^2	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($J \cdot mol^{-1}$)	R^2	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($J \cdot mol^{-1}$)	R^2	D_0 ($cm^2 \cdot s^{-1}$)	E_A ($J \cdot mol^{-1}$)
BZP	2.3×10^{-04}	31.16	0.9899	1.6×10^{-02}	38.68	0.9817	1.5×10^{-05}	20.36	0.9999	2.2×10^{07}	86.9
DPBD	3.9×10^{-07}	25.70	0.9851	nm	nm	nm	5.5×10^{-05}	39.26	0.9938	1.6×10^{07}	86.9
Uvitex® OB	2.3×10^{03}	78.43	0.9971	nm	nm	nm	1.5×10^{-05}	38.8	0.8617	1.6×10^{06}	86.9

nm: no migration

Partition coefficient

Likewise D_E, $K_{P/F}$ is also a crucial parameter for migration modelling and it depends on several factors. $K_{P/F}$ is the concentration ratio of the migrant in the polymer and in the foodstuff when the equilibrium state is achieved (Ossberger, 2009).

Migrants are often lipophilic substances, therefore migration is usually higher into foodstuffs with high fat content. Also, lipids are able to enter the polymer, inducing swelling (Riquet, Wolff, Laoubi, Vergnaud and Feigenbaum, 1998; Sanches Silva, Cruz, Sendón García, Franz and Paseiro Losada, 2007b). As such, in these kind of foods, $K_{P/F}$ is usually lower (lower $K_{P/F}$ indicates higher concentration in packaging material and higher concentration in food). In this work results (Table 5.3), $K_{P/F}$ is clearly higher when cooked ham (selected foodstuff with higher fat content) is used. Meanwhile, the differences between $K_{P/F}$ are not so obvious when red wine and tomato sauce are used despite the does not has any fat while tomato sauce has 1.3 % lipid content.

Comparison between experimental and predicted D values

As it was said in chapter 1, there are numerous mathematical models to predict the diffusion of migrants. Regrettably, many of the models that are available are not practical. This is because they require parameters and the determination of these parameters would be less accurate and even more laborious and expensive, than the traditional migration experimentation required by the regulation. In order to avoid this problem, a simple and practical equation (Eq. 1.6) that does not requires experimental data and that relates D_P with the model substance MW, type of polymer matrix and temperature was developed (Piringer, 1994; Brandsch *et al*, 2002). With equation, overestimated values of D_P (D^*_P) were calculated (table 5.5)

Table 5.5. *Predicted diffusion coefficient in the polymer (D^*_P)*

Temp. (ºC)	D^*_P ($cm^2 \cdot s^{-1}$)		
	BZP	**DPBD**	**Uvitex® OB**
20	7.22×10^{-09}	5.34×10^{-09}	5.28×10^{-10}
40	7.04×10^{-08}	5.21×10^{-08}	5.15×10^{-09}
60	5.23×10^{-07}	3.86×10^{-07}	3.82×10^{-08}

By comparing the theoretical diffusion coefficient D^*_P (table 5.5) with the experimental D_E (table 5.3), it is possible to observe that D_E is always lower than D^*_P, indicating that Eq 1.6 successfully predicts an (intentionally) overestimated diffusion coefficient for these experimental conditions.

As it was done previously, it is also possible to calculate D_0 and E_A for these values. These theoretical D_0 and E_A are shown in table 5.4. In order to better these values understand, the diffusion coefficients are graphically represented in Fig. 5.1 for the temperature range of 0 – 100 ºC. In this figure, it is assumed that linearity remains for temperatures out of the experimental temperature range.

As it was said previously, for the experimental range of temperatures, D^*_P is an overestimation of D_E. yet, by observing Fig. 5.1, the slope of the curves is different (as a consequence of different E_A) meaning that, if linearity remains, at some temperatures the real diffusion coefficient of the LDPE-food system will surpass the prediction calculated with Eq. 1.6. In most cases, this will not bring any worries regarding food safety, because it would only happen at temperatures far below real use conditions. For example, in cooked ham, this would only happen at temperatures below -56.2 and -45.7 ºC for DPBD and Uvitex® OB, respectively; in tomato sauce, this happens at -7.7 ºC for BZP, -38.5 ºC for DPBD and -117.2 for Uvitex® OB; in. Nevertheless, in our results, there some cases that require more caution. In the case of BZP in cooked ham and in red wine, D^*_P is lower than D_E at temperatures that will likely correspond or are very close to real storage

conditions, like refrigeration at 4 ºC (temperatures below 12.3 ºC for cooked ham and below 2.3 ºC for red wine). For these two cases, mainly in the case of the migration of BZP from LDPE into cooked ham, it is possible that the amount of migrant transferred into the foodstuff is actually higher than the amount predicted.

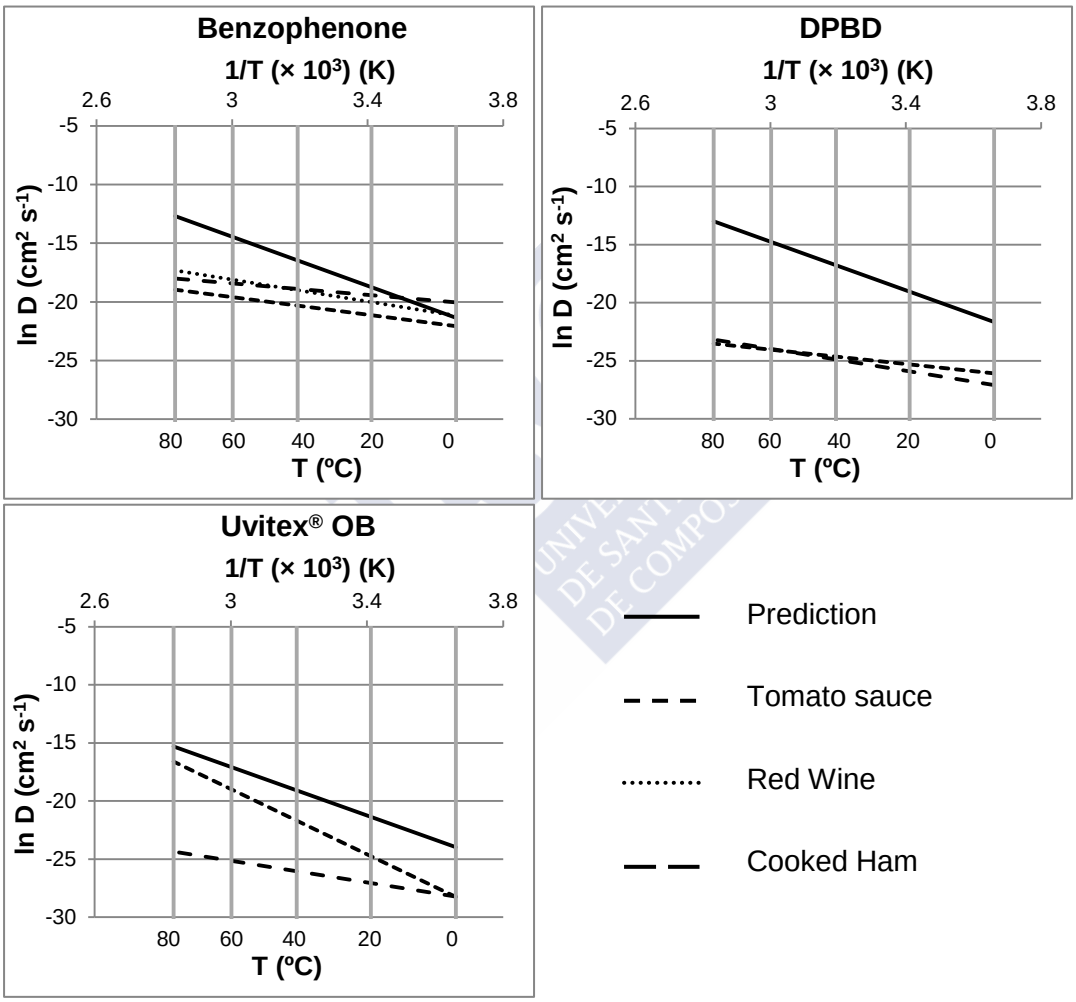

Figure 5.1. *Comparison between D and D^*_P in the range of 0 to 80 ºC*

5.4 Conclusion

In this work, two essential parameters (partition and effective diffusion coefficients) for migration modelling where calculated. This experimental data is important to better understand the migration of components of packaging materials into real foodstuff, where data is still limited. This paper also useful to validate the migration model employed, which demonstrated a good fit between predicted and experimental data.

The migrating behaviour shows dependence of various factors like the model substance properties, type of foodstuff used and storage conditions used. D_E is higher when the temperature increases and when the MW of the migrant decreases; $K_{P/F}$ is higher for BZP and lower for Uvitex® OB, for the same foodstuff. Higher migration was also observed when lower MW substances and foodstuffs with higher fat content were used.

The experimental effective diffusion coefficient (D_E) was always lower than the predicted diffusion coefficient in the film (D^*_P) at the experimented temperatures, meaning that D^*_P may be a useful and yet easy to apply tool for regulatory purposes.

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6

Study of the diffusion behaviour of model substances from LDPE packaging into real foodstuff

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Abstract

Integrated in the European project FACET, the migration behaviour of three model substances from LDPE into solid foodstuffs was calculated under different temperatures and essential migration parameters were calculated. This information is extremely useful to better understand the migration phenomenon due to the lack of bibliographic support about these parameters, especially when real foodstuff is used. The selected model migrants and foodstuffs were chosen so that different chemical properties and food compositions were reunited.

Migration is an interaction between packaging and foodstuff that consists on the mass transfer of the food contact material (FCM) components into packed foodstuff. This phenomenon cannot be completely stopped and, due to the nature of some FCM components, these may have negative effects in the consumer's health. As such, specific legislation to limit consumer's exposure to these components was created.

The migration parameters effective diffusion coefficient and partition coefficient were successfully calculated for the proposed LDPE/food systems by fitting experimental data with a proposed mathematical model.

Keywords: Migration; modelling; diffusion coefficient; partition coefficient; LDPE; food packaging;

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6.1 Introduction

For the last decades, the use of plastics as food packaging materials has been increasing significantly. This is due that several advantages that plastics have over other packaging materials: ample range of flexibility, size and shapes, thermal sealability and price (Hernandez-Muñoz, Catalá and Gavara, 2001). At the same time, there has also been a growing concern about consumer's health safety related to the transfers of substances from packaging materials into foodstuff. This is associated to the increased use of plastic packages in contact with food, longer contact times between food and package, greater diversity food contract materials (FCM), larger number of substances used in the production of these materials (new raw materials, additives, etc.) and also due to improved knowledge about harmful effects of some FCM components (Dainelli *et al*, 2008; Poças and Hogg, 2007).

There may be several food-packaging interactions like, permeation, sorption and migration. This last one is the one that requires more concern from the health safety point of view due to the toxic effect that is inherent to the constituents of the plastic packaging materials (Sanches Silva, Cruz Freire, Franz and Paseiro Losada, 2008). Migration strongly depends on several factors, such as storage time-temperature conditions and physicochemical characteristics of the polymer, food and migrant. Some of the characteristics involved in migration phenomena are the migrant molecular weight, its initial concentration in the polymeric film, polarity, diffusivity and chemical structure; food-packaging interactions, their structure and properties (Ossberger, 2009; Canellas, Aznar, Nerín and Mercea, 2010). These substances that migrate into food are mainly low weight molecules such as additives, residual monomers, reaction by-products and impurities that are not attached to the polymeric matrix and, as such, are able to move freely within it (Helmroth, Rijk, Dekker and Jongen, 2002).

To determine the level of migration, a set of rules and time-temperature conditions are described in the current European legislation (European Commission, 2011). These conditions were chosen so that they replicate a worst-case scenario, which are the most extreme foreseeable conditions that a specific food packaging material is subjected to. In these tests, real foodstuffs are substituted by food simulants that represent different categories of food (e.g. acetic acid 3 %, simulant B, is used to test foods with acidic characteristics like vinegar). The use o food simulant is meant to reduce the analytical work because foods are extremely complex matrices. Even so, this is a very time-consuming and costly task (Sanches Silva, Sendón García, Cooper, Franz and Paseiro Losada, 2007). A different approach to test several samples of different packaging-food (or food simulant) combinations at different time-temperature conditions are to proceed to estimation of migration through mathematical modelling. With key parameters properly measured, these models are useful tools to calculate the migration of substances from package into foodstuffs. Generally recognised diffusion models supported by scientific evidence that are created to overestimate real migration are already allowed in the European legislation since 2002 to confirm compliance between plastic materials intended to be in contact with food and the current legislation. However, when non compliance is verified, the extent of migration must always be determined experimentally. (European Commission, 2011)

As stated above, migration modelling requires key parameters, namely diffusion and partition coefficients (D and $K_{P/F}$, respectively). D is linked to the kinetic of the transfer process and measures the rate at which the model substance migrates from the packaging material into the food; and $K_{P/F}$ represents the thermodynamic equilibrium and it is described as the ratio of migrant concentration in the polymeric material and its concentration in the food (or food simulant) when equilibrium state is achieved. (Granda-Restrepo *et al*, 2009; Tehrany and Desobry, 2004).

In this study, three model substances were tested as study subjects for the migration process between low density polyethylene (LDPE) films into real solid foodstuffs. The three selected migrants are diphenyl phthalate (DPP), 2-isopropylthioxanthone (ITX) and butylated hydroxitoluene (BHT), whilst for the selected foodstuffs three highly consumed foods in Europe were chosen: Gouda cheese, turkey ham and cooked ham. The selected model migrants are used in the plastic industry with diverse applications. DPP is a plasticizer used in the manufacturing of plastics to impart flexibility and workability during production and to the end product. It may also be used in paints, lacquers, adhesives, cardboard, lubricants and fragrances (Tuteja, Khan and Sundararajan, 2005). ITX is used as photoinitiator in UV inks applied to paper and plastic that may be used as food packaging materials (Gallart-Ayala, Moyano and Galceran, 2008). BHT it is a low weight and fat-soluble organic compound with antioxidant properties that is used in polymers to protect them from thermal degradation. It is also used food additive (E321) or in the production of cosmetics, rubber, paints and others (Torres-Arreola, Soto-Valdez, Peralta, Cardenas-López and Ezquerra-Brauer, 2007).

With the acquired data, the experimental data was fitted with a proposed deterministic mathematical model based on Fick's second law of diffusion and the parameters D and $K_{P/F}$ were calculated. These parameters are essential to predict the migration phenomenon and are meant to be used by 7th Framework EU funded project Flavourings, Additives and food Contact materials Exposure Task (FACET, 2008). The final objective of this project is not only to develop a mathematical modelling tool that allows the estimation of migration from FCM into foodstuffs. Instead, FACET project is also meant to provide a validated software program for modelling food intake and estimate consumer's exposure to chemical through ingestion of contaminated food.

6.2 Experimental:

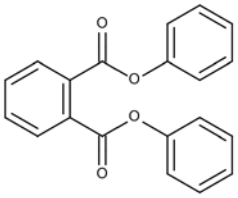
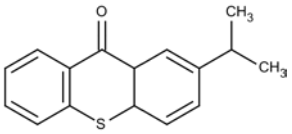
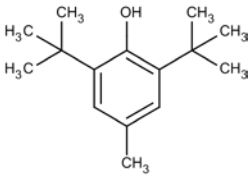
6.2.1 Chemicals and standard solutions:

In this work, three substances selected were selected as possible model migrants: diphenyl phthalate (DPP; purity ≥ 99%), isopropylthioxanthone (ITX; mixture of 2- and 4-isomers; purity ≥ 99%) and butylated hydroxitoluene (BHT; purity 97%). All the migrants were supplied by Sigma-Aldrich (Steinheim, Germany). Physicochemical properties and chemical structure of these migrants are summarized in Table 6.1.

Ethanol (EtOH; absolute for analysis) and acetonitrile (ACN; for liquid chromatography) were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA, USA). Sodium Azide (purity 99%; CAS no. 26628-22-8) was supplied by Sigma Aldrich (Steinheim, Germany).

Stock solutions of each substance with an accurately known concentration of approximately 500 $\mu g \cdot ml^{-1}$ were prepared with EtOH. From these stock solutions, calibration standard solutions containing all three model substances were prepared with EtOH. The concentration range of these solutions is 0.05-10 $\mu g \cdot ml^{-1}$. Solutions were stored at 4 ºC and protected from light.

Table 6.1. *Structure and physicochemical properties the used migrants*

Chemical structure			
Name	Dyphenyl phthalate (DPP)	Isopropylthioxanthone (ITX)	Butylated hydroxytoluene (BHT)
CAS no.	84-62-8	5495-84-1	128-37-0
Molecular weight	318.3	254.4	220.4
Boiling point (ºC)	255 [a]	398.9 [b]	265 [a]
Melting point (ºC)	73 [a]	77.5 [a]	71 [a]
Log P (o/w)	3.18 [a]	5.113 [b]	5.36 [a]

Data acquired from Scifinder® database; [a] experimental values; [b] estimated values

6.2.2 Foodstuff

Three different solid foodstuffs were chosen for this study: Gouda cheese; cooked ham; and turkey ham. These foodstuffs were selected for being highly consumed in Europe and for having different diffusion behaviour due to the differences in their composition (different fat, protein and water content, different consistency).

Gouda cheese has high fat content (27.44 %), is high in protein (24.94 %) and still with considerable water content (41.46 %). From the three foodstuffs, it is the one with higher migration potential. Turkey ham and cooked ham have a similar composition (approx. 72 % water and 18-19 % protein). The main difference between these two foods is the fat content, being 3.80 % for turkey ham and 7.62 % for cooked ham (USDA database).

6.2.3 Plastic films:

In this work, a LDPE plastic film produced by Gaiker was used as source of migrants. This polymer was highly additivated with DPP, ITX and BHT during the production by a extrusion process. The film is 412.1±15.1 μm thick and it has a concentration of 505.4±86.9 $mg \cdot L^{-1}$ of DPP, 1214.5±116.7 $mg \cdot L^{-1}$ of ITX and 863.7±153.0 $mg \cdot L^{-1}$ of BHT.

6.2.4 Migration conditions

The LDPE film additivated with DPP, ITX and BHT was cut in 10 cm^2 samples. A total of 10 samples were cut for each food/temperature combination. Each polymer sample was weighted and placed between two food slices with the same dimensions and weighted earlier. A small amount of sodium azide was added to the food sides that were not in contact with the polymer to avoid microbial growth during the experiment.

The setting foodstuff-LDPE-foodstuff was them taken and wrapped in aluminium foil and then sealed in a polyamine/polyethylene bag to prevent food from drying. The samples were then stored at three different temperatures (20, 40 and 60 ºC) for predetermined times, except for turkey that was stored at onlt two temperatures, 20 and 40 ºC. The storing times were:

- 20 ºC – 2; 4; 8; 12; 24; 48; 96; 168; 240; 360 (h)
- 40 ºC – 1 ; 2; 4; 8; 12; 24; 48; 96; 168; 240 (h)
- 60 ºC – 0.5; 1 ; 2; 4; 8; 12; 24; 48; 96; 168 (h)

6.2.5 Migrant quantification

Determining the specific migration experimentally is a tedious and complex process that consumes great amounts of time and resources, and optimized and validated methods are required. Unfortunately these do not exist for all food, migrants and packaging materials, due to the vast number of these items (Sanchez-Silva, Pastorelli, Cruz, Simoneau, Castanheira and Paseiro-Losada, 2008). Foodstuff is a complex matrix from the analytical point of view that requires sometimes elaborated methods when extracting/determining migrant transferred into it or that can be a possible source of many interferences and/or sample losses. In order to simplify, it is possible to use food simulants (acid acetic 3%, EtOH 10%, EtOH, 20%, EtOH 50% and vegetable oil) under controlled time-temperature conditions instead of real foodstuff (Sanchez Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007; European Commission, 2011). Even so, problem may appear, especially when using vegetable oil as food simulant.

In order to avoid these possible setbacks and also to reduce the work load, a different approach was taken when determining the quantity of migrant transferred into the food. When performing migration studies, the quantity of migrant transferred into the food is normally calculated after extracting it from the food. In this work, the amount of migrant remaining in the LDPE (after contact with the food) was calculated after a simple extraction procedure. Then, the amount that migrated into the foodstuff was calculated as the difference between the initial and final concentration of the model substances in the FCM.

The extraction from the additivated LDPE film consisted on taking the plastic that had been in contact with the selected foodstuffs, clean it carefully with paper tissue (to remove residues of food present in the polymer surface) and place it in a flask filled with 50 ml of ethanol. The flask was tightly closed and placed in an

oven at 70ºC. After 1 hour, the flask was taken and, when at room temperature, an aliquot of the extracting solution was taken and analysed by HPLC.

After this extraction, migrant residues on the polymer surface were removed by quick immersion in fresh EtOH. A second extraction was performed in the films by submitting the polymer to the same conditions as in the first extraction.

6.2.6 High performance liquid chromatography

A Agilent (Waldbronn, Germany) system consisting of with a HP 1200 liquid chromatograph coupled to a quaternary pump, an autosampler, a column thermostat system, a diode array detector (DAD) and a fluorescent detector (FLD). The DAD was continuously performing a scan in the range of 190 to 400 nm. For data acquisition, HP ChemStation® chromatographic software was used.

Chromatographic separation was performed with a Kromasil C18 column (250 × 3.20 mm internal diameter, 5 µm particle size) (Phenomenex, Barcelona, Spain) at 30 ºC. DPP and BHT were both detected by the DAD at 205 nm, while ITX was detected by fluorescence at 250 nm and 410 nm (emission and excitation wavelengths, respectively).

The mobile phase, with a constant flow rate of 0.5 ml·min^{-1} during the entire analysis, was composed by two solvents: Milli-Q water and ACN. The elution method starts with 40 % Milli-Q water and 60 % ACN for 1 minute. Afterwards, the percentage of ACN increases gradually until it reaches 100 % at 17 minutes and then the elution remains isocratic for 3 minutes, until the end of the HPLC method. The volume of sample injected was 20 µl. The model migrants were identified by comparison of their retention times and UV spectrum with those of previously injected standards using the same HPLC conditions.

6.3 Results and discussion

6.3.1 Calibration

To proceed to the calibration of the HPLC method, series of solutions containing DPP, ITX and BHT were analyzed. A total of 8 calibration points with concentrations ranging from 0.05 to 10.0 $\mu g \cdot mL^{-1}$ were used. Each solution was analyzed 3 times and the average area of the peaks was taken. Calibration line equation was calculated by plotting the average peak areas against the concentration of the standard solutions.

A good regression line was obtained for each substance ($R^2 \geq 0.9995$), demonstrating that the method is suitable for the quantification of these three substances. The calibration lines add a slope of 174.9 and an intercept of 0.532 for DPP, 262.8 and 1.52 for ITX, and 162.0 and 74.0 for BHT. The limit of detection, established accordingly to the guidelines of the American Chemical Society (ACS, 1980) of each substance was 0.025 $\mu g \cdot ml^{-1}$ or lower.

6.3.2 Determination of key parameters

The parameters D_E and $K_{P/F}$ related to the migration of three model substances (DPP, ITX and BHT) from a LDPE film into real food samples was determined by fitting the experimental data with the proposed mathematical model (Eq. 1.2 and 1.3) and the respective RMSE was calculated.

These fundamental parameters are shown in table 6.2 and the migration kinetics are shown in Fig. 6.1.

Table 6.2. *Calculated migration key parameters (D_E and $K_{P/F}$) and RMSE*

Food	Subst.	Temp (ºC)	D_E ($cm^2 \cdot s^{-1}$)	$K_{P/F}$ calc	RMSE (%)
Gouda Cheese	DPP	60	5.50×10^{-09}	3.26	5.90
		40	3.78×10^{-09}	1.31	5.72
		20	1.03×10^{-09}	1.63	8.61
	ITX	60	8.32×10^{-09}	6.43	7.66
		40	4.63×10^{-09}	3.31	3.53
		20	9.07×10^{-10}	2.33	1.35
	BHT	60	4.00×10^{-09}	10.67	6.22
		40	1.90×10^{-09}	4.41	2.21
		20	5.69×10^{-10}	4.17	2.36
Cooked Ham	DPP	60	9.61×10^{-10}	2.07	3.84
		40	8.49×10^{-10}	3.19	3.89
		20	4.62×10^{-10}	3.62	6.43
	ITX	60	8.96×10^{-11}	16.55	2.67
		40	3.45×10^{-11}	19.98	1.72
		20	1.66×10^{-11}	24.55	3.43
	BHT	60	4.66×10^{-10}	25.30	2.50
		40	6.04×10^{-11}	26.35	3.18
		20	7.74×10^{-12}	50.68	5.38
Turkey Ham	DPP	60	-	-	-
		40	8.58×10^{-10}	3.05	3.40
		20	3.41×10^{-09}	5.04	5.38
	ITX	60	-	-	-
		40	5.85×10^{-11}	17.69	1.88
		20	1.28×10^{-11}	24.04	1.69
	BHT	60	-	-	-
		40	5.93×10^{-11}	19.83	1.73
		20	4.30×10^{-11}	24.29	1.77

6.3.3 Partition coefficient

Partition coefficient is a parameter related to the relative solubility of a substance in the polymer and in the foodstuff when equilibrium is achieved (Sanchez Silva, Cruz Freire, Sendón García, Franz and Paseiro Losada, 2007).

D depends on several factors: nature of the packaging material, chemical structure and polarity of the migrants, types of foodstuff (Ossberger, 2009). Accordingly to Gandek *et al* (1989), $K_{P/F}$ is not significantly affected by temperature. Also, to ensure that $K_{P/F}$ is calculated accurately, it is needed that the system has achieved equilibrium. Unfortunately, sometimes, this is not possible because long storage times are required and changes or spoilage in food may occur. In addition to this fact, there are also other factors that may interfere with the determination of $K_{P/F}$, i.e. variations in the plastic (variation in thickness, migrant not homogenously distributed in the polymer film) or in the food matrix (thickness, differences in the homogeneity of food matrix). Also, when the migrant is almost complete or almost inexistence, small variations in the cuantification of the migrant may influence greatly the determination of $K_{P/F}$. For these reasons, these values should be considered with caution.

Observing the calculated $K_{P/F}$ values of the selected substances on table 6.2, it is lower for DPP and higher for BHT ($K_{P/F}$ is inversely proportional to the concentration of the migrant in foodstuff, meaning that when this parameter value is lower indicates a higher relative concentration of migrant in food). This is can be explained with the partition coefficient oil/water (log P (o/w) is 3.567 for DPP; 5.113 for ITX; and 5.36 for BHT). LDPE is apolar, so more polar substances (with higher log P o/w) have more affinity to the foodstuff fat content and have a tendency to leave the polymer into the foodstuff; less polar substances (lower log P o/w), have an inferior affinity to the foodstuff, so it tends to remain in the

polymer. This also elucidates why ITX and BHT, two substances with similar log P (o/w), have similar $K_{P/F}$.

When comparing this parameter in the different foodstuffs, $K_{P/F}$ values are also lower when the additivated LDPE film is in contact foods with higher fat content (fat content in cheese > cooked ham > turkey ham). Here, the same reasoning as above may be applied: migrants with higher log P (o/w) have higher affinity to foods with higher fat content.

6.3.4 Effective diffusion coefficient

Effective dffusion coefficient (D_E) represents the kinetic of the transfer process of the migrant from a highly concentrated material into another material with lower concentration, that is to say, the velocity of the flux of migrant from packaging into the foodstuff.

Unlikely to the partition coefficient, the diffusion coefficient greatly depends of the temperature, where higher temperatures lead to higher D_E values, as a result of the faster migration rate. This fact is confirmed by our results shown in table 6.2. Without any exception, for a same migrant-food system, D_E achieved at 60ºC is always the higher one, while at 20ºC is always the lowest.

As it has been said previously, by using Eq. 1.4, it is possible to use this correlation between temperature and D_E to predict D at temperatures different from the ones used experimentally. The D_0, E_A and R^2 are represented in table 6.3.

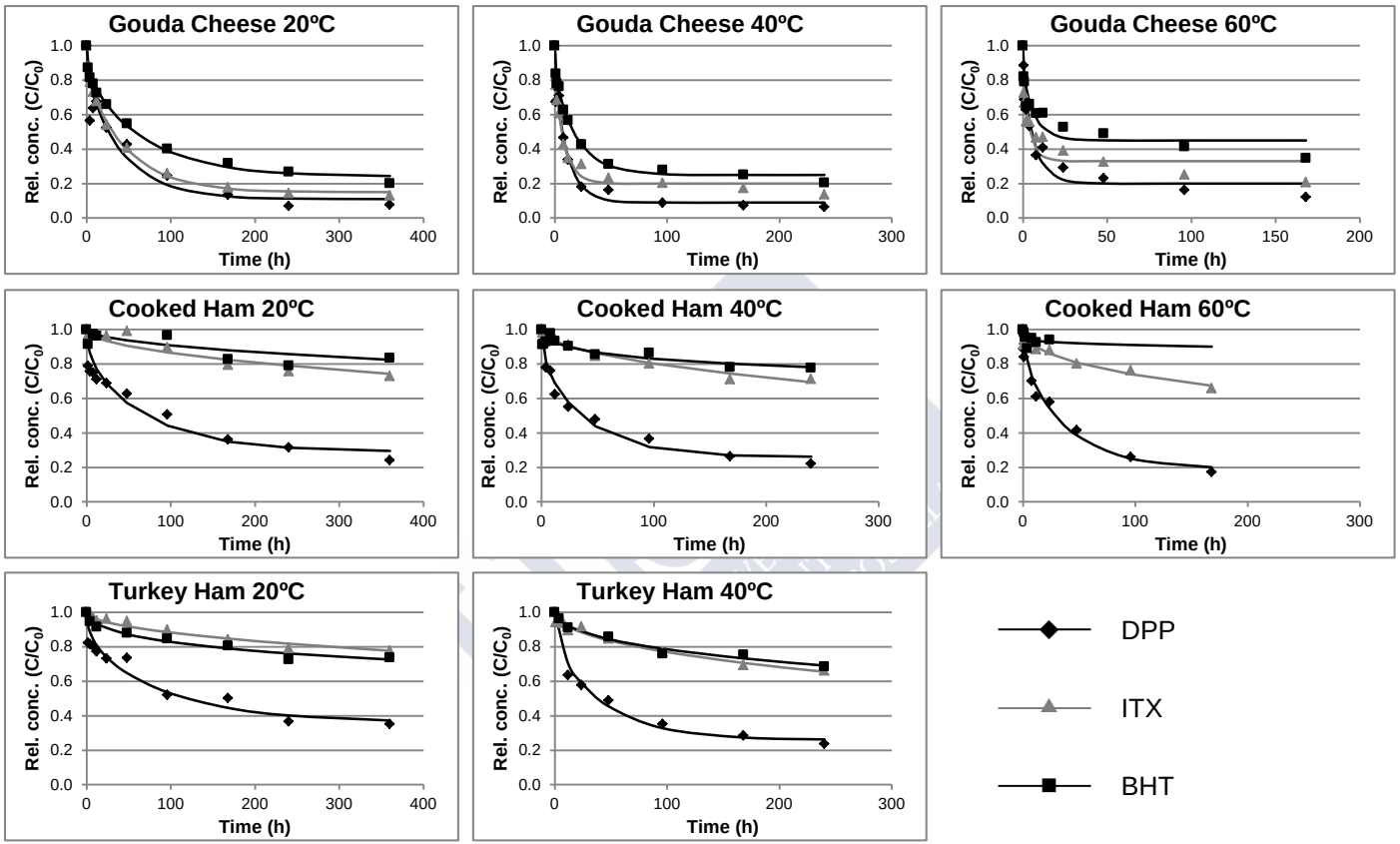


Figure 6.1. *Relative concentration of model substances in LDPE during the experiment*

If diffusion coefficient between BHT additivated LDPE film and Gouda cheese at 5 ºC is calculated with eq. 6 (and assuming the linearity would remain for temperature below 20 ºC), then $D_{BHT,\ Gouda\ cheese,\ 5\ ºC} = 2.49\times10^{-10}$ $cm^2\cdot s^{-1}$. Cruz, Sanchez Silva, Sendón García, Franz and Paseiro Losada (2008) determined experimentally D for these same conditions (6.34×10^{-11} $cm^2\cdot s^{-1}$). Despite the apparent difference between both values (D_E calculated in this work is approximately 4 times higher), it cannot be assumed that these values are inaccurate. It is important to notice that the LDPE and Gouda cheese used in both works was not exactly the same and small differences in their constitution can justify the observed differences in the results.

Table 6.3. *Calculated pre-exponential factors and activation energies*

Food	Subst.	D_0 ($cm^2\cdot s^{-1}$)	E_A ($kJ\cdot mol^{-1}$)	R^2
Gouda Cheese	DPP	1.55×10^{-3}	34.4	0.9280
	ITX	1.28×10^{-1}	45.4	0.9488
	BHT	7.15×10^{-3}	39.7	0.9904
Cooked Ham	DPP	2.40×10^{-7}	15.1	0.8962
	ITX	1.84×10^{-5}	34.0	0.9870
	BHT	4.75×10^{3}	83.1	0.9988
Turkey Ham	DPP	6.51×10^{-4}	35.2	-
	ITX	2.59×10^{-1}	57.8	-
	BHT	6.59×10^{-9}	12.3	-

This coefficient is also affected by other factors linked to the food and model substance nature. Regarding the food nature, the factor that has a stronger influence over D_E is the food fat content and, as it might be seen in table 6.2,

higher D_E values were found when Gouda cheese (fat content = 27.44 %) was used. For turkey ham and cooked ham, differences for the same migrant and storage conditions were not significant, acting in accordance with the low differences in fat content of these foodstuffs (turkey ham: 3.80 %; cooked ham: 7.62 %) respect to Gouda cheese. Sometimes D_E in turkey ham is higher than cooked ham, which has twice the fat content than the turkey ham, indicating that other food related factors are interfering with this parameter.

Regarding the migrant characteristics, the one that mostly affects D_E is the molecular weight (MW). This relationship between migrant MW has already been proposed by many author, such as Limm and Hollifield (1996), Piringer (1994) or Brandsch, Mercea, Piringer (2000). Although, according to these authors, the MW is the most important migrant related factor affecting the diffusivity of the model substance for most of cases, our results indicate that there is another factor or a group of factors that may overcome the effect of the migrants MW in their diffusion from polymer into foodstuff.

6.4 Results and discussion

The present paper supports the validation of the used a Fick's Second Law of Diffusion based mathematical model, where D_E is used instead of D_P, as a tool for migration modelling. Also, instead of determining the transferred amount of migrant from the food, this was calculated after extraction from the additivated polymer, allowing to simplify the process, reduce interference and avoiding changes in the extraction procedure every time that a new foodstuff is used. A

good correlation between experimental and predicted data was found and essential key parameters for migration modelling were successfully calculated.

Results show that migration depends on the food nature (mainly fat content) and on the migrant physicochemical characteristics. Unlike the information found in the bibliography, the migration rate (represented by D_E) was faster when the molecular weight of the model substance was higher. This implies that, although the molecular weight is the most important factors, there are other factors that have an important role in this entire process.

Following an Arrhenius type equation, a good correlation between D_E at different temperatures was found, permitting the prediction of D_E at temperatures different from the ones used experimentally.

The data acquired in this work is useful to better understand the mechanism that have an effect on mass transfer of packaging material components into real foodstuff and will be used by the European project FACET. This project has the task to develop a migration model to estimate migration from food packaging materials into foodstuff (under real-use conditions) and to provide valid software to estimate food chemical intake through a deterministic and probabilistic model.

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7

Effect of detergents in the release of bisphenol A from polycarbonate baby bottles

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Abstract

Bisphenol A (BPA) is a monomer crucial for the production of polycarbonate (PC). Recently, it has been verified that BPA is able to migrate from PC baby bottles into food simulants and, although this is a controversial issue, numerous studies indicate that BPA may have an effect on the human health. Consequently, BPA safety has been an increasing alarming topic.

In this work, we tested 5 different detergents (2 hand and 3 machine dishwashing detergents) and bleach to verify if they may have an effect on the depolymerization of PC baby bottles, by increasing the BPA release from the PC. High-performance liquid chromatography (HPLC) with fluorescence detection (FLD) and gas-chromatography coupled to mass spectrometry (GC-MS) were used to quantify and identify BPA, respectively. Of all the 5 detergents tested, only with one was not detected a BPA concentration higher than the control. In the worst case, with a hand detergent (detergent B), BPA levels detected were about 500 times higher than the control and the concentration kept high even after the PC samples were rinsed three times. This did not happen with any other of the tested detergents. In the case of bleach, the BPA released was reduced to not detectable levels, while the bleach was in contact with the PC.

Keywords: Bisphenol A; detergent; polycarbonate; depolymerization; migration.

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7.1 Introduction

Polycarbonate (Fig. 7.1) is a thermoplastic polymer with high transparency, low weight and high heat and impact resistance. Due to these characteristics and the fact that it can be easily worked, PC has been widely used. PC may be found at compact disks, drinking bottles, eyeglasses lenses, mobile phones, plastic food containers, houseware, as a replacement of glass in several products, such as baby feeding bottles, and many other products [1, 2].

The key building block of PC is bisphenol A (BPA; 2,2'-bis(4-hydroxyphenyl)propane; CAS No. 80-05-7) (Fig. 7.1). This monomer is one of the highest production-volume chemical in the world [3] and it is present in quite a lot of products that people are in contact with every day. In 2003, more than 2 million metric tons were produced globally and its demand increases up to 6-10% every year. The main application of BPA production is for the manufacture of epoxy resins and, especially, of PC (21% and 72%, respectively) [4]. BPA can also be used as additive in PVC films [5].

Several studies have already confirmed that BPA release from PC baby bottles takes place [6; 7; 8]. This release is not primarily due to migration, because the BPA diffusion from the PC to the beverage is very low. Rather than that, most of the BPA released results from PC degradation [9]. Accordingly to some authors, BPA may have an effect on the human health.

Data obtained from in vitro assays shown that BPA has weak estrogenic activity and there is some concern about how it may affect the endocrine system by mimicking estradiol. This may happen even in the presence of very low BPA doses [10; 11]. There are also in vivo studies, made in mammals (mainly in mice and rats) that point to other adverse effects caused by BPA. Some examples can be cited: increase in hormonally mediated cancers, such as prostate and breast

cancer; abnormalities in reproductive organs; a decline in semen quality; early sexual maturation in females; metabolic disorders including insulin resistant diabetes and obesity; and neurobehavioral problems such as autism and attention deficit hyperactivity disorder [12-16]. These data, plus the fact that detectable levels of BPA are present in the urine of more than 90% of the US population [17], makes the BPA an alarming issue.

However, BPA safety aspects are increasingly controversial. Agencies such as the US Food and Drugs Administration (FDA), European Food Safety Authority (EFSA) and Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), supported by the plastic industry, argue that, whenever BPA is used accordingly to the existing legislation, it does not represent any risk to human health when used in food contact materials [18-20]. On the other hand, the Health Canada chose to take a preventive approach and concluded that BPA should be considered as a substance that may constitute a danger to human life or health [21]. For this reason, Canada has announced the preparation of regulations to ban the importation, advertising and sale of PC baby bottles containing BPA. Measures to reduce the amount of BPA that is released into the environment will also be taken. Several U.S. states, such as California, are already preparing legislation to take similar provisions.

To wash baby bottles, as well as other childcare products, it is usual to use ordinary dishwashing detergents and hot water. Depending on people habits, the baby bottles washing procedure can include hand wash, machine wash, brushing, soaking in hot soapy water, etc.

The aim of this work was to test the effect of different detergents on PC baby bottles, to see if commercially available detergents may increase the BPA release due to a depolymerization effect on the material. To do this, migration tests, at controlled times and temperatures, were performed by incubating PC samples with

several detergent solutions. The PC samples were then rinsed with distilled water and migration tests were repeated, this time with distilled water. This last procedure was repeated for three times in order to check if the BPA release would continue after the removal of the residual detergent. After each incubation, the solutions were analyzed directly by HPLC-FLD.

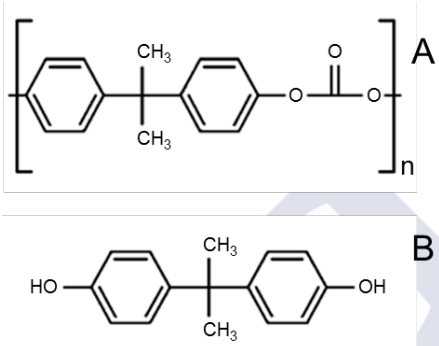

Figure 7.1. *Molecular structure of PC (A) and BPA (B)*

7.2 Experimental

7.2.1 Chemicals and reagents

Bisphenol A, 99+% (Aldrich-Chemie, Steinheim, Germany); water obtained using Milli-Q apparatus from Millipore Ireland B.V. (Carringtwohill, Ireland); sodium hypochlorite, sol. reagent grade 10-13% (Sigma-Aldrich, Steinheim, Germany).

7.2.2 Samples

Baby bottles made of PC, bought in a local supermarket, were chosen to be our study samples. These baby bottles were produced in China.

The samples were prepared by cutting each baby bottle in several pieces with an area of 10 cm^2. The cuts were made vertically seeking that the pieces were representative of the different bottle zones. For each test, three pieces were used and each one was cut in half in order to fit the headspace vials where they were incubated.

The detergents were also bought in a local supermarket and two of them were hand dishwashing detergents (detergents A and B), while the other three were machine dishwashing detergents (detergents C, D and E). The detergent concentration used was 10 $g \cdot L^{-1}$. Bleach was also tested. Instead of using a commercial brand we used a sodium hypochlorite solution, at a concentration of 1 $g \cdot L^{-1}$. These concentrations were chosen because they seem to be close to the real use concentrations.

In parallel with samples, a control was prepared by subjecting a PC sample to the same conditions but without using any detergent or bleach solution. In this case only distilled water was used all the time.

7.2.3 Apparatus and conditions

High performance liquid chromatography with fluorescence detection (HPLC-FLD) was performed using a Hewlett-Packard HP 1100 chromatograph equipped with a diode array detector and a fluorescence scanning detector arranged in

series. The conditions used for determination of BPA by HPLC-FLD are listed in table 7.1.

Gas chromatography with mass spectrometry (GC-MS) was carried out using a THERMO Finnigan TraceGC ultra chromatograph with a TraceDSQ mass detector and a TriPlus AS automatic injector. The conditions used for identification of BPA by GC-MS are listed in table 7.2.

The oven used for the incubation tests was a Memmert, model ULE 400.

Table 7.1. Conditions and instrument settings used for determination of BPA by HPLC-UV-FLD

Pump	Hewlett-Packard Quaternary HP 1100 pump		
Injection volume	20 µl		
Column temperature	25 ºC		
Detector	Fluorescence HP 1100: scan excion range 200-280 nm Ultraviolet HP 1100: scan range 190-400 nm		
Degasser	HP 1100 Vacuum degasser		
Wavelength	Fluorescence: excitation 225 nm, emission 305 nm Ultraviolet: 225 nm, Ref. 360 nm		
Column	Kromasil C18 25 x 0.36 cm I.D., 5 µm particle size		
Mobile phase	A: Milli-Q water; B: Acetonitrile		
Flow	0.5 ml·min^{-1}		
Gradient	Time (min)	% A	% B
	0.00	70.00	30.00
	2.00	70.00	30.00
	30.00	20.00	80.00
Software	HP ChemStation		

Table 7.2. Conditions and instrument settings used for identification of BPA by GC-MS

Constant flow	0.8 ml·min^{-1}
Carrier gas	He
Column:	
Dimensions	30 m x 0.250 mm
Film surface	1 µm
Liquid phase	DB-5MS
Injector temperature	225ºC
Split mode	1:30
Injection volume	1.0 µl
Column temperature program	Isocratic (250ºC for 20 min)
Mass spectrometer, THERMO instrument Finnigan TraceDSQ	
Interphase temperature	200ºC
Electron energy	70 eV
Electron multiplier	1504 V
Full-scan	m/z 50-400
SIM mode	m/z 213 (base peak), 228
Electron impact	
Spectrum library	Wiley
Software	XCalibur Home Page version 1.4 SRI, Windows XP

7.2.4 Identification of BPA by GC-MS

Successive dilutions of a standard 1000 mg·L^{-1} BPA solution in ethanol were analyzed in full-scan and single ion monitoring (SIM) modes, according the

conditions mentioned in table 7.2, in order to determinate the limit of detection of BPA by GC-MS. The limit of detection was achieved when the BPA peak was three times higher than the noise level.

7.2.5 HPLC-FLD method validation

Calibration of the HPLC was made by injecting, in triplicate, seven BPA standard solutions in water. The solution concentrations were 10, 5, 1, 0.5, 0.1, 0.05, 0.01 $mg \cdot L^{-1}$. The calibration line of concentrations versus chromatographic peak areas was obtained by linear regression.

The limit of detection was calculated by successive dilutions of a BPA standard solution until the BPA peak-to-noise ratio was 3:1.

Precision measurement was estimated by injecting 10 times a 0.1 $mg \cdot L^{-1}$ BPA solution in water and calculating the relative standard deviation.

7.2.6 Study of the effect of detergents on Polycarbonate

Baby bottles are always washed up and sterilized before being used. The following experiment was designed under extreme conditions to know the effect of detergents and bleach on PC.

Three baby bottle pieces (total area 30 cm^2) were immersed in a 15 ml solution of detergent or bleach in distilled water. The concentration of these solutions was 10 $g \cdot L^{-1}$ for the detergents and 1 $g \cdot L^{-1}$ for the bleach.

After being immersed in the solutions, the samples were incubated at 120 ºC, for one hour. Once the incubation was over, samples were allowed to cool down at room temperature for 30 min and 0.6 ml of the solution was taken for HPLC analysis in order to evaluate if the detergent was able to affect the PC during the washing and soaking procedure in very hot water. Then the samples were left further at 25 ºC for 5 days in the same solution. Once again, a small volume of solution was taken for HPLC analysis. The purpose was to see if the depolymerization process caused by detergent would occur, even at low temperatures.

To find out if the release of BPA would stop after the detergent was removed, the PC pieces were washed in distilled water and immersed again, but this time in distilled water, for one hour at 120 ºC. Next, they were left cooling down at room temperature for 30 min, an aliquot of the water was taken for HPLC analysis and the PC pieces were washed again in distilled water. This process was repeated once a day, for two more days. The objective was to verify if the rinsing procedure was enough to stop or to diminish the eventual BPA release.

7.3 Results and discussion

7.3.1 Identification of BPA by GC-MS

With the GC-MS analytical method described in Table 7.2, BPA elution time was 10.52 min (Fig. 7.2). BPA was identified by comparing the mass spectra of the peak obtained in the samples with that of a BPA standard solution and also comparing against the XCalibur library.

The limit of detection in full-scan mode was 50 mg·L^{-1}, while in SIM mode (m/z=213, corresponding to the demethylated fragment of BPA, and m/z=228, corresponding to BPA [5]) was 0.5 mg·L^{-1}.

7.3.2 HPLC method performance

The calibration line obtained by injecting seven standard solutions with a concentration ranging 0.01-10 mg·L^{-1} showed a good linearity correlation (R^2 > 0.9999) and is represented by the following equation:

$$y = 2399.4x - 21.385$$

The limit of detection determined was 5.0 µg·L^{-1} and was obtained by running successive dilutions of a BPA stock solution until the BPA peak-to-noise ratio was 3:1.

The estimated relative standard deviation was 1.53%, after injecting ten times a 0.1 mg·L^{-1} BPA solution.

With the HPLC analytical method described in Table 7.1, BPA elution time was 16.20 min (Fig. 7.3).

7.3.3 BPA release from baby bottles

The results values of the migration of BPA from PC baby bottles into the different solutions assayed in this work are shown in table 7.3.

Migration of BPA was detected in the control sample, when distilled water was used (without any detergent or bleach). BPA concentration found was 0.109 $mg \cdot L^{-1}$, after the first incubation period.

Comparing all detergents, detergent A is the only one that seems to decrease the BPA release from PC, being BPA concentrations 4-5 times lower than those found in the distilled water, during the firsts two measurements. For this detergent, after rinsing the PC samples, the levels of BPA became close to that found in the control. In the case of bleach, although higher pH values should increase the BPA release [10], the levels were not detectable while the PC was in contact with the sodium hypochlorite solution (first two measurements). However, once the sodium hypochlorite is removed from the samples by rinsing, and the samples are incubated in distilled water only, BPA release is observed.

Detergents B, C, D and E showed a higher release of BPA than that observed in the control. BPA levels were approximately 500, 28, 7 and 283 times higher, respectively, than the concentration found in the control in the first measurement. After the PC samples were rinsed, BPA levels diminished to levels comparable to those found in the control either after the first rinsing (for detergents D and E) or the third rinsing (for detergent C). For detergent B, even though there was a visible decrease of the BPA levels during the rinsing procedures, significant concentrations (31.0-44.6 $mg \cdot L^{-1}$) could still be detected. These concentrations were 1680-1860 times higher than those found in the control samples.

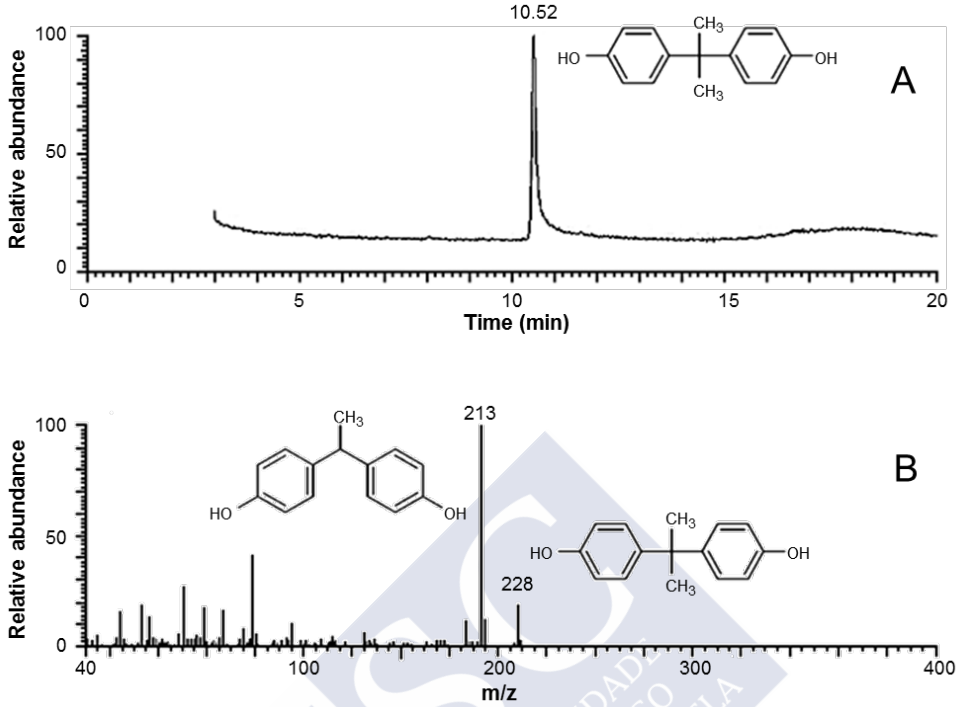

Figure 7.3. *GC-MS Chromatogram of an acetonitrile extract of a PC baby bottle sample (A) and a mass spectrum of the 10.52 min peak of the chromatogram (B)*

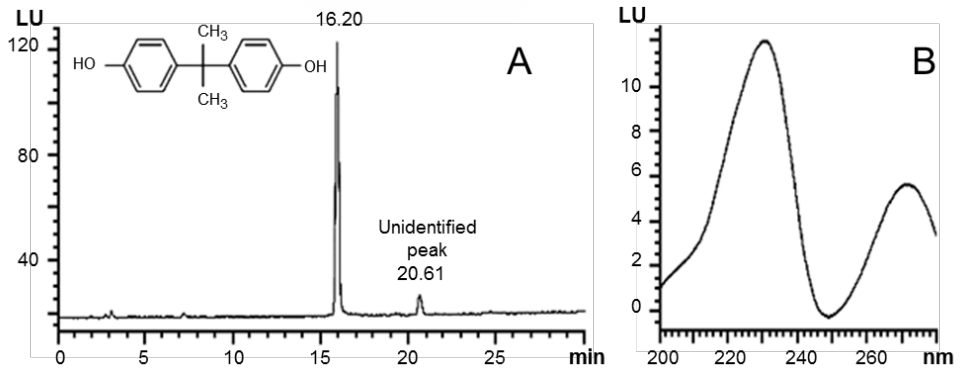

Figure 7.3. *HPLC chromatogram obtain from detergent B first measure, after being 1:100 dilution (A) and fluorescence excitation spectrum (λem=305 nm) of the BPA peak (B)*

Table 7.3. *Migration of BPA from PC baby bottle samples into the detergent solutions, determined by HPLC-FLD*

Solutions	Conc. ($g \cdot L^{-1}$)	Type of detergent	No. of exp. (n)	Concentration of BPA ($mg \cdot L^{-1}$)				
				120ºC, 1h	+ 25ºC, 120h	+ 120ºC, 1h (1st rising with distilled water)	+ 120ºC, 1h (2nd rising with distilled water)	+ 120ºC, 1h (3rd rising with distilled water)
Dist. water		-	3	0.109	0.086	0.024	0.030	0.018
Det. A	10	HW	3	0.022	0.020	0.012	0.027	0.018
Det. B	10	HW	3	54.8	67.0	40.4	44.6	31.0
Det. C	10	MW	3	3.03	2.65	0.111	0.065	0.030
Det. D	10	MW	3	0.809	0.645	0.016	0.020	0.014
Det. E	10	MW	3	30.9	33.3	0.037	0.050	0.041
Bleach	1	-	3	n.d.	n.d.	0.045	0.043	0.020

n.d. – not detected; HW – hand wash detergent; MW – machine wash

7.4 Conclusion

BPA safety issue is presently a controversial issue and this monomer presence in a large percentage of the population indicates us that better knowledge about BPA origin is necessary.

These results also demonstrate that the BPA detected in the detergent solutions (except for the detergent A solution) does not result from diffusion,

because BPA concentrations are much higher than that found in the control. Instead, BPA is primarily a product from a PC degradation process originated by the contact between detergent and PC at high temperatures.

In the current study was demonstrated that dishwashing detergents may increase the BPA release while the detergent is in contact with the PC. The BPA concentration decrease immediately near to control levels after rinsing the PC samples (0.018-0.041 $mg \cdot L^{-1}$, after the third rinsing), except for one of the cases tested (detergent B), where, although there is a visible decrease on BPA concentration, the levels detected are still very high (approximately 31.0 $mg \cdot L^{-1}$, after the third rising).

Further studies are needed in order to better understanding of the BPA release from PC mechanism that we are facing, and also would be need to perform more tests in the conditions required by the European Council Directive 82/711/EEC [22], simulating real-use conditions.

Acknowledgements

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8

Effect of amines in the release of bisphenol A from polycarbonate baby bottles

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Abstract

The key block for the production of polycarbonate (PC) is bisphenol A (BPA). Recent studies have proven that this monomer is able to migrate from PC baby bottles into food simulants and, although this is a polemical subject, numerous investigations indicate that BPA may have an effect on the human health. For these reasons, BPA safety regarding human exposure has recently become an alarming issue.

Amines are a class of chemicals which are present in foodstuffs, such as milk. For this reason PC baby bottles, while being used, are continuously in contact with several amines, some of which are able to cause PC aminolysis, resulting in the release of BPA.

In this work, 16 substances (14 with amine groups and 2 with amide groups) were tested in order to verify if they were able to increase BPA release by increasing PC depolymerization. High-performance liquid chromatography (HPLC) with fluorescence detection (FLD) and gas chromatography coupled to mass spectrometry (GC-MS) were used to quantify and identify the BPA, respectively. Although most of the substances tested did not increase the release of BPA from PC, some of them had a significant effect and high levels of this monomer were measured in the solutions. Of all of the amines tested that originating the worst case of BPA release was 1,4-diaminobutane. Also known as putrescine, 1,4-diaminobutane is a biogenic amine that results from protein degradation and it may be present in milk. In this case, BPA concentration in the solution was more than 5000 times the level found in the control sample.

Keywords: Bisphenol A; amines; polycarbonate; migration; depolymerization

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8.1 Introduction

Bisphenol A (BPA; 2,2'-bis(4-hydroxyphenyl)propane; CAS No. 80-05-7) (Fig. 8.1) is one of the highest production-volume chemicals in the world and it is present in several products that are use every day by the consumers. In 2003, more than two million metric tons were produced throughout the entire world and its demand increases up to 6–10% every year (Lang *et al*., 2008). This monomer is produced mostly to manufacture epoxy resins and, predominantly, PC (21% and 72%, respectively) (Fig. 8.1) (Chapin, 2007). Due PC characteristics (toughness, light weight, transparency, high impact and temperature resistance), this material is very frequent in household and food contact products, such as food containers, baby bottles, kitchen appliances, plastic bottles, toys, mobile phones and countless other products (Nérin, Fernández, Domeño, & Salafranca, 2003; Wong, Leo, & Seah, 2005). BPA may also be found in PVC films, where it is used as an additive (López-Cervantes & Paseiro-Losada, 2003).

It has already been demonstrated that BPA is released from PC baby bottles into food simulants (Biles, McNeal, Begley, & Hollifield, 1997; Brede, Fjeldal, Skjevrak, & Herikstad, 2003; Maragou, Makri, Lampi, Thomaidis, & Koupparis, 2007). This release is not primarily due to a migration process, because BPA diffusion from the PC to the beverage is very low. Rather than a diffusion process, most of the BPA leaching results from PC degradation (Bierdermann-Brem & Grob, 2009).

According to some authors, BPA may be harmful to human health. Data obtained from in vitro assays indicates that BPA has weak estrogenic activity and there is some apprehension about how it may affect the endocrine system by mimicking estradiol, even when very low doses are present (Krishnan, Stathis, Permuth, Tokes, & Feldman, 1993; Laws, Carey, Ferrell, Bodman, & Cooper,

2000; Simal-Gándara, Paz-Abuín, & Ahrné, 1998). There are also in vivo studies, made in mammals (mainly in mice and rats) that call attention to other adverse effects caused by BPA. Some examples can be cited: increase in hormonally mediated cancers, such as prostate and breast cancer; abnormalities in reproductive organs; a decline in semen quality; early sexual maturation in females; metabolic disorders including insulin resistant diabetes and obesity, and neurobehavioral problems such as autism and attention deficit hyperactivity disorder (Farabollini, Porrini, & Dessì-Fulgheri, 1999; Gupta, 2000; Howdeshell, Hotchkiss, Thayer, Vandenbergh, & Vom Saal, 1999; Saal & *et al*., 2007; Timms *et al*., 2005). Knowing this and the fact that detectable levels of BPA are present in the urine of more than 90% of the US population (Calafat *et al*., 2005), the BPA issue deserves special attention.

This matter is, even though, increasingly polemical. Agencies such as the US Food and Drug Administration (FDA), European Food Safety Authority (EFSA) and Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), supported by the plastic industry, argue that, whenever it is used in congruence with the existing legislation, BPA does not represent any risks to human health when used in food contact materials (Aguilar *et al*., 2008; Philbert *et al*., 2008; Scientific Committee on Toxicity, Ecotoxicity and the Environment, 2002). On the other hand, the Health Canada, deciding to take a precautionary approach, concluded that BPA should be considered as a substance that may constitute a danger to human life or health (Environment Canada, 2008). For this reason, Canada announced that it will proceed with drafting regulations to ban the importation, advertising and sale of polycarbonate baby bottles, being the first country to take such measures. These regulations are also projected in order to reduce the amount of BPA that is released into the environment. Some U.S. states, such as California, are already preparing legislation to adopt similar provisions.

Amines are a wide group of substances occurring naturally in almost every food, such as fish, meat, vegetables, fruit, wine, cheese, beer and also milk (Silla Santos, 1996). Therefore, it can be considered as normal that amines are constantly in contact with PC baby bottles (as well as other PC materials). The use of amines has already been studied as a possible way to recycle plastics, because PC can be degraded by aminolysis, resulting in the release of BPA (Hata, Goto, Yamada & Oku, 2002). In this paper, several substances, including amines and amides, are assayed in order to find out which of them induce a higher BPA release from PC. To do this, migration tests were done, at controlled times and temperatures, by incubating PC samples with selected substances solutions. The PC samples were then rinsed with distilled water and migration tests were repeated, this time with just distilled water. At the end of each incubation period, the solutions were directly analyzed by HPLC-FLD.

8.2 Experimental

8.2.1 Chemicals and reagents:

Bisphenol A, 99+% (Aldrich-Chemie, Steinheim, Germany); water obtained using Milli-Q apparatus from Millipore Ireland B.V. (Carringtwohill, Ireland); sodium hydroxide, pellets GR for analysis (Merck Chemicals, Darmstad, Germany); 1,3-phenylenediamine, 99+% (Aldrich-Chemie, Steinheim, Germany); 1,3-cyclohexanebis(methylamine), 99% (Aldrich-Chemical, Milwaukee, USA); 1,4-diaminobutane, 99% (Aldrich-Chemical, Milwaukee, USA); 4-aminophenyl sulfone, 98% (Aldrich-Chemie, Dorset, England); ε-caprolactam, 99+% (Aldrich-Chemie,

Steinheim, Germany); erucamide, >85% (Aldrich-Chemical, Milwaukee, USA); ethylenediaminetetraacetic acid, 99% (Panreac, Barcelona, Spain); hexamethylenetetramine, 99% (Aldrich-Chemie, Steinheim, Germany); L-glutamine, 99+% (Sigma, St. Louis, USA); L-lysine, 99+% (Sigma, St. Louis, USA); m-xylylenediamine; melamine, 99% (Aldrich-Chemical, Dorset, England); N,N-dimethylethanolamine, 99% (Aldrich-Chemie, Steinheim, Germany); N,N,N',N'-tetramethylethylenediamine, 99+% (Merck, Darmstadt, Germany); trimethylamine, ~45% (Fluka, Steinheim, Germany); urea, >99,5% (Merck, Darmstadt, Germany).

These chemicals structures are represented in Fig. 8.1.

8.2.2 Samples

PC baby bottles, bought in a local supermarket, were chosen to be our samples in this work. These baby bottles were produced in China. It was not possible to get enough samples from the same lot, so samples of two different lots were used.

The samples were prepared by cutting each baby bottle in several pieces, each one with an area of 10 cm^2. The cuts were made vertically searching that the pieces were representative of all different baby bottle zones. For each test, three pieces were used and then they were cut in half in order to fit the headspace vials where they were incubated.

8.2.3 Apparatus and conditions

High performance liquid chromatography with fluorescence detection (HPLC-FLD) was performed using a Hewlett-Packard HP 1100 chromatograph equipped

with a diode array detector and a fluorescence scanning detector arranged in series. The conditions used for determination of BPA are listed in table 8.1.

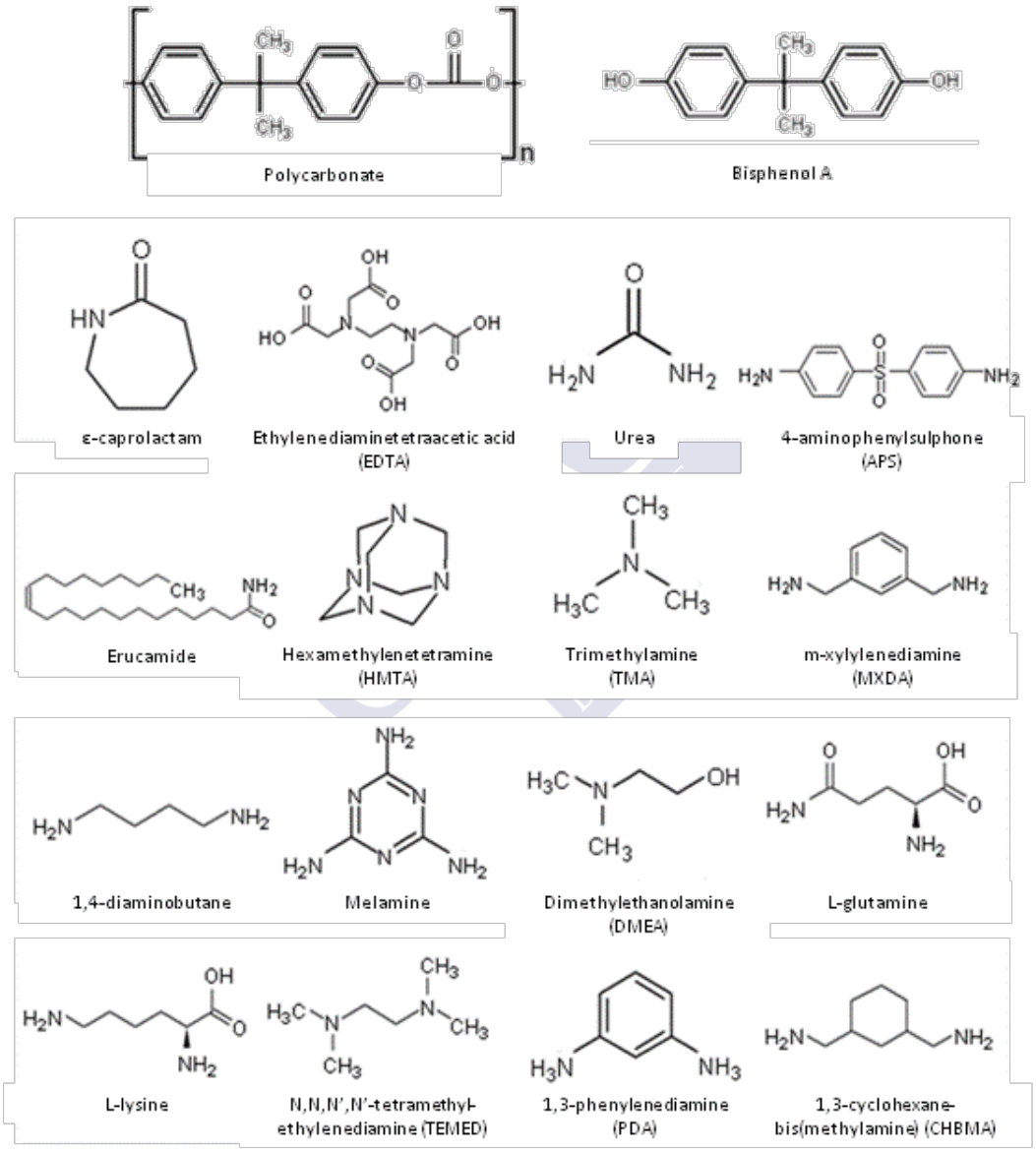

Figure 8.1. *Molecular structure of PC, BPA and of the substances (amines and amides) used in this work*

Gas chromatography with mass spectrometry (GC-MS) was carried out using a THERMO Finnigan TraceGC ultra chromatograph with a TraceDSQ mass detector and a TriPlus AS automatic injector. The conditions used for identification of BPA are listed in table 8.2.

The oven used for the incubation tests was a Memmert, model ULE 400.

Table 8.1. *Conditions and instrument settings used for determination of BPA by HPLC-UV-FLD*

Pump	Hewlett-Packard Quaternary HP 1100 pump		
Injection volume	20 µl		
Column temperature	25 ºC		
Detector	Fluorescence HP 1100: scan excion range 200-280 nm Ultraviolet HP 1100: scan range 190-400 nm		
Degasser	HP 1100 Vacuum degasser		
Wavelength	Fluorescence: excitation 225 nm, emission 305 nm Ultraviolet: 225 nm, Ref. 360 nm		
Column	Kromasil C18 25 x 0.36 cm I.D., 5 µm particle size		
Mobile phase	A: Milli-Q water B: Acetonitrile		
Flow	0.5 $ml \cdot min^{-1}$		
Gradient	Time (min)	% A	% B
	0.00	70.00	30.00
	2.00	70.00	30.00
	30.00	20.00	80.00
Software	HP ChemStation		

8.2.4 Identification of BPA by GC-MS

Successive dilutions of a standard 1000 mg·L^{-1} BPA solution in ethanol were analyzed in full-scan and single ion monitoring (SIM) modes, according the conditions mentioned in table 8.2.

The limit of detection of BPA identification was determined by injecting successive dilutions of a BPA standard solution until the BPA peak-to-noise ratio was 3:1.

8.2.5 HPLC-FLD method validation

Calibration line was obtained by linear regression of concentrations against chromatographic peak areas of injected BPA standard solutions with concentration ranging 0.01 – 10.0 mg·L^{-1}.

The limit of detection was calculated by injecting successive dilutions of a BPA standard solution until the height of the BPA peak was approximately three times higher than the noise level.

Precision measurement was estimated by running a 0.1 mg·L^{-1} BPA solution in water 10 times and calculating the relative standard deviation.

8.2.6 Study of the effect of amines in Polycarbonate

Three baby bottle pieces (total area 30 cm^2) were immersed in a 15 ml solution of amine in water, with a concentration of 600 mg·L^{-1}. The amines tested were: 1,3-phenylenediamine, 1,3-cyclohexanebis(methylamine), 1,4-

diaminobutane, 4-aminophenyl sulfone, ethylenediaminetetraacetic acid, hexamethylenetetramine, L-glutamine, L-lysine, m-xylylenediamine; melamine, N,N-dimethylethanolamine, N,N,N',N'-tetramethylethylenediamine, trimethylamine and urea. The amides tested were ε-caprolactam and erucamide.

Table 8.2. *Conditions and instrument settings used for identification of BPA by GC-MS*

Constant flow		0.8 ml·min^{-1}
Carrier gas		He
Column:	Dimensions	30 m x 0.250 mm
	Film surface	1 µm
	Liquid phase	DB-5MS
Injector temperature		225ºC
Split mode		1:30
Injection volume		1.0 µl
Column temperature program		Isocratic (250ºC for 20 min)
Mass spectrometer, THERMO instrument Finnigan TraceDSQ		
Interphase temperature		200ºC
Electron energy		70 eV
Electron multiplier		1504 V
Full-scan		m/z 50-400
SIM mode		m/z 213 (base peak), 228
Electron impact		
	Spectrum library	Wiley
	Software	XCalibur Home Page version 1.4 SRI, Windows XP

After being immersed in the solutions, the samples were incubated at 120 ºC, for one hour. Once the incubation ended, the samples were left cooling down at room temperature for 30 min and 0.6 ml of the solution was taken for HPLC analysis. The aim was to evaluate if the amines were able to depolymerize the PC while being in contact at high temperature. Then the samples were left further at 25 ºC for 5 days in the same solution. Once again, a small volume was taken for HPLC analysis. This was meant to verify if the possible depolymerization due to the presence of amines would occur, even at low temperatures.

To determine if the release of BPA would stop or diminish after the amines solutions were removed, the PC pieces were rinsed with distilled water and immersed again, but this time in distilled water, for one hour at 120 ºC. Next, they were allowed to cool down at room temperature for 30 min, a water sample was taken for HPLC analysis and the PC pieces were washed again in distilled water. This process was repeated once a day, for two more days. The purpose of this step was to verify if the rinsing procedure was enough to stop or to diminish the eventual BPA release.

Accordingly to Bierdermann-Brem and Grob (2009), polycarbonate is labile in alkali environment. For this reason, an increment of BPA may be an effect caused by the high pH observed in some of the solutions used and not an effect caused directly by the amine itself. To prevent this, a test was made under the conditions cited above but replacing the amines by NaOH solution. For this test, only the first measure was made and the NaOH solution pHs were 8.8, 9.8, 10.8 and 12.1.

8.3 Results and discussion

8.3.1 Identification of BPA by GC-MS

BPA elution time was 10.52 min (Fig. 8.2). BPA was identified by comparing the mass spectra of the peaks obtained in the samples with that of BPA standard solution and also comparing against Wiley library.

The limit of detection attained in full-scan mode was 50 $mg \cdot L^{-1}$, while in SIM mode (at m/z 213, corresponding to the demethylated fragment of BPA) was 0.5 $mg \cdot L^{-1}$.

8.3.2 HPLC method performance

The calibration line obtained by injecting seven standard solutions with a concentration ranging from 0.01-10 $mg \cdot L^{-1}$ showed a good linearity correlation (R2>0.9999) and is represented by the following equation:

$$y = 2399.4x - 21.385$$

The limit of detection determined was 5.0 $\mu g \cdot L^{-1}$ and was attained by running successive dilutions of a BPA stock solution until the BPA peak height was three times the noise level.

The relative standard deviation, estimated by injecting ten times a 0.1 $mg \cdot L^{-1}$ BPA solution, was 1.53%.

BPA elution time was 16.20 min (Fig. 8.3).

8.3.3 BPA release from baby bottles

The values of BPA migration from PC baby bottles into the solutions assayed in this study are shown in table 8.3.

BPA was detectable in all samples, even in the control samples, where the simulant was distilled water (97 $\mu g \cdot L^{-1}$ for lot A control sample and 47 $\mu g \cdot L^{-1}$ for lot B control sample, in the first measurement). For 9 of the 16 substances tested (ε-caprolactam; ethylenediaminetetraacetic acid; 4-aminophenyl sulfone; urea; erucamide; melamine; L-glutamine; L-lysine; 1,3-phenylenediamine), no apparent effect on BPA release was detected. In these cases, the measured BPA amount was similar or even lower than the values found in the control sample (22-137 $\mu g \cdot L^{-1}$ in the first measurement). In the case of the sample in contact with the N,N,N',N'-tetramethylethylenediamine solution, the concentration of BPA detected was about 15.7 times higher than the control sample, but when compared with the values obtained for the NaOH solution with pH=10.8 we observe that the BPA depolymerization is a consequence of the higher pH value of this amine solution (Bierdermann-Brem & Grob, 2009). The same applies to N,N-dimethylethanolamine and hexamethylenetetramine.

The samples that were brought in contact with the others 4 amines solutions showed an increment of BPA release that cannot be explain exclusively by pH effect alone. When compared with their corresponding control samples, m-xylylenediamine and trimethylamine were responsible for an increment on BPA

release of 20.8 and 49.6 times, respectively. Even though these were significant values, a higher effect was found for 1,3-cyclohexanebis(methylamine) and 1,4-diaminobutane, being the BPA concentrations measured in the solutions 3853.0 and 5363.9 times higher than the control samples, respectively.

During the rinsing procedures, there is a notorious decrease of BPA leaching into the solutions, but levels of BPA higher than those found in the control samples were still observed. For 1,4-diaminobutane and 1,3-cyclohexanebis(methylamine), the high presence of BPA after the rinsing may be explained by the fact that, during the first incubation, there was an extreme elevated BPA formation and the rinsing was not enough to remove it successfully. In the case of trimethylamine, the BPA level reduction after rinsing with water was less noted. The extension of the BPA release during the rinsing procedures may be due to the fact that trimethylamine has a low molecular weight, thus being possible that the molecule was trapped in the polymeric net and its elimination by rinsing was not effective. This way, even after washing three times the PC samples, some trimethylamine was still available to depolymerize the PC.

8.4 Conclusion

BPA presence in the urine of a large fraction of the world population and the doubts about its safety regarding human health show that is important to acquire more knowledge about the origins of this monomer.

The current work demonstrates that the BPA detected in the amine solutions does not result from a diffusion mechanism, because BPA concentrations are much higher than those found in the control samples. Instead, BPA is primarily a

Table 8.3. *Migration of BPA from PC baby bottle samples into the amine solutions, determined by HPLC-FLD*

	Solutions	pH	No. of exp. (n)	Concentration of BPA (mg·L^{-1})				
				120ºC, 1h	+ 25ºC, 5 days	+ 120ºC, 1h (1st rising with distilled water)	+ 120ºC, 1h (2nd rising with distilled water)	+ 120ºC, 1h (3rd rising with distilled water)
Lot A	Dist. Water	6.9	3	0.097	0.091	0.094	0.036	0.044
	ε-caprolactam	6.8	3	0.079	0.080	0.041	0.043	0.025
	EDTA	5.0	3	0.022	0.022	0.017	0.065	0.025
	Urea	8.9	3	0.042	0.045	0.025	0.047	0.024
	APS	7.2	3	0.062	0.059	0.021	0.027	0.071
	Erucamide	7.5	3	0.041	0.046	0.021	0.022	0.020
	HMTA	9.8	3	0.262	0.299	0.054	0.034	0.023
	TMA	10.4	3	4.803	5.171	10.681	3.023	2.022
	MXDA	10.3	3	2.014	2.578	0.367	0.065	0.061
	DAB	10.5	3	520.296	492.573	35.839	15.857	7.943
	Melamine	8.8	3	0.137	0.147	0.042	0.069	0.052
	DMEA	10.6	3	0.896	0.774	0.108	0.055	0.032
Lot B	Dist. water	6,9	3	0.047	0.045	0.033	0.020	0.022
	L-glutamine	6.2	3	0.030	0.024	0.026	0.024	0.023
	L-lysine	5.8	3	0.024	0.021	0.021	0.019	0.022
	TEMED	10.6	3	0.740	0.701	0.239	0.139	0.144
	PDA	7.1	3	0.046	0.041	0.034	0.026	0.026
	CHBMA	11.4	3	181.091	180.236	25.795	8.538	6.085
	NaOH	8.8	3	0.165	-	-	-	-
	NaOH	9.8	3	0.249	-	-	-	-
	NaOH	10.8	3	0.583	-	-	-	-
	NaOH	12.1	3	5,479	-	-	-	-

EDTA – ethylenediaminetetraaceti acid; APS - 4-aminophenyl sulfone;
HMTA – hexamethylenetetramine; TMA - trimethylamine;
MXDA – m-xylylenediamine; DAB – 1,4-diaminobutane;
DMEA – N,N-dimethylethanolamine; TEMED – N,N,N,N-tetramethylethylenediamine
PDA – 1,3-phenylenediamine; CHBMA – 1,3-cyclohexanebis(methylamine)

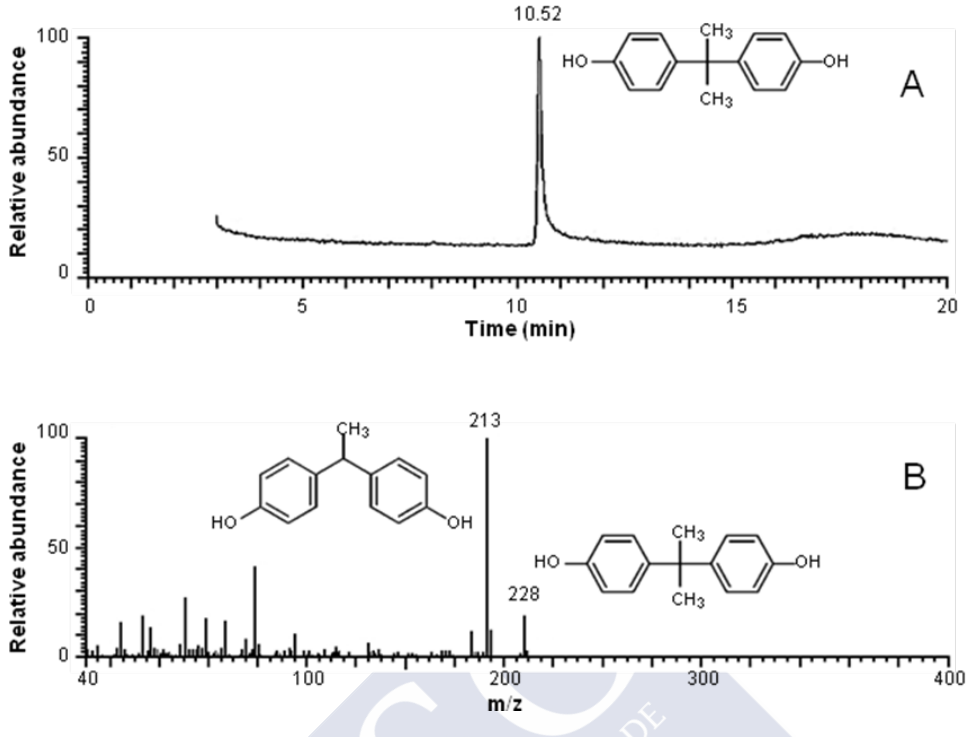

Figure 8.2. *GC-MS Chromatogram of an acetonitrile extract of a PC baby bottle sample (A) and a mass spectrum of the 10.52 min peak of the chromatogram (B)*

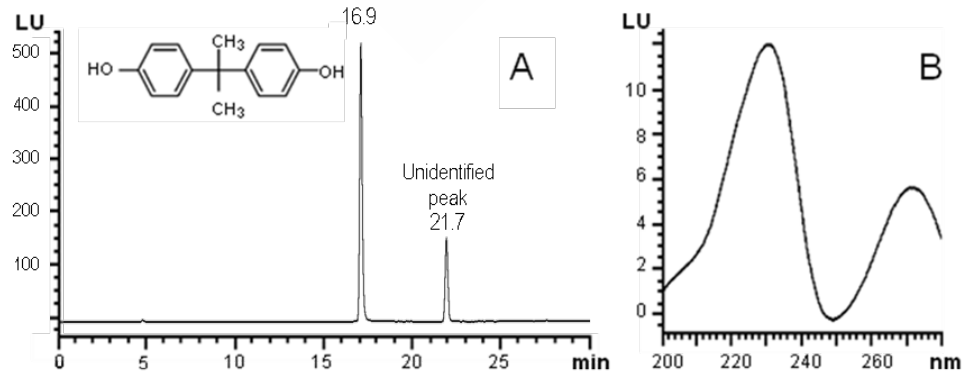

Figure 8.3. *HPLC chromatogram obtain from TMA first measure (A) and fluorescence excitation spectrum (λem=305 nm) of the BPA peak (B)*

product of a PC degradation process originated by the contact between the material and certain amines at high temperatures.

Of all the substances tested, the ones that produced higher values of BPA while in contact with PC were aliphatic diamines with low molecular weights. They were primary, secondary or tertiary amines.

1,4-diaminobutane and trimethylamine, two biogenic amines that gave positive results in this study, may be detected in milk at low concentrations. If migration tests are performed with conventional simulants (accordingly to the European Council Directive 85/572/EEC of 19 December 1985, the milk simulant is ethanol 50% v/v), the effect of the amine in the PC baby bottles is not taken in account and this could lead to an underestimation of the actual BPA release.

Further studies, using amines, especially those that can be found in foodstuffs and that are more probable to enter in contact with baby bottles, are needed in order to better understand the mechanism of BPA release from PC. This is important in order to choose the correct testing conditions that best simulate what is happening under real conditions of use and, this way, to fit what is established in the European Council Directive 82/711/EEC.

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9 Conclusiones generales

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Seguidamente se puntualizan las principales conclusiones extraídas de los trabajos que forman esta Tesis Doctoral.

Relativo a las cinéticas de migración LDPE-alimento

Primera:

Se ha desarrollado una metodología para determinar las cinéticas de migración desde plásticos a alimentos basada, a diferencia de la tradicional, en determinar la cantidad residual en el polímero. El análisis es más sencillo y rápido que cuando se procede a la determinación del migrante en el alimento.

Segunda:

El coeficiente de difusión efectivo y el coeficiente de partición se calcularon para diversos migrantes en varios sistemas compuestos por LDPE y diversos alimentos. El modelo matemático utilizado para la determinación de estos dos parámetros demostró ser apropiado, ya que se ajusta eficazmente a los puntos experimentales.

Tercera:

El uso de distintos alimentos, migrantes y temperaturas para estudiar el proceso de migración permitió determinar algunas de las propiedades que tienen mayor influencia sobre los parámetros citados anteriormente.

De una manera general, los factores que influyen sobre el coeficiente de difusión efectivo en el sistema LDPE/alimento son: la temperatura, el contenido en grasa de los alimentos y la masa molecular del migrante. El coeficiente de difusión

efectivo aumenta cuando aumenta la temperatura y el contenido en grasa, y cuando disminuye la masa molecular del migrante.

Por otro lado, el coeficiente de partición de sistema LDPE/alimento depende de la naturaleza del migrante y de la naturaleza del alimento. Este parámetro aumenta cuando disminuye la afinidad del migrante hacia el alimento. Cuando las sustancias son más lipofílicas (como es el caso de las sustancias estudiadas en esta tesis, que tienen un log (o/w) superior a 3) el coeficiente de partición es menor cuando se utilizan alimentos con una elevada proporción grasa/agua. Además, este parámetro no se ve influenciado significativamente por la temperatura.

Cuarta:

Mediante la utilización de la ecuación de Arrhenius (Ec. 1.5), se demostró buena linealidad entre los coeficientes de difusión efectivos, determinados experimentalmente a lo largo del rango de temperaturas estudiadas, y se calcularon dos parámetros (la energía de activación y el factor pre-exponencial) asociados a la cinética de cada sistema en los que ocurre la migración. Con estas dos variables, es posible estimar coeficientes de difusión efectivos a temperaturas distintas de las usadas en este estudio.

Quinta:

Los coeficientes de difusión efectivos (D_E) se compararon con los coeficientes de difusión en el polímero calculados empíricamente a través de la de ecuación de Piringer (D^*_P; Ec. 1.6). Estos coeficientes de difusión teóricos en el polímero siempre han sido superiores a los coeficientes de difusión efectivos

experimentales para el rango de temperaturas estudiado. Esto indica que, desde el punto de vista de la seguridad alimentaria, esta ecuación puede ser utilizada como una primera aproximación para el valor del coeficiente de difusión efectivo, incluso en las situaciones donde la migración ocurre muy rápido. Sin embargo, los resultados indican que este método no es viable para todas las temperaturas y, por debajo de una temperatura (que va a depender de cada caso), el coeficiente de difusión en el polímero estimado con la ecuación de Piringer dejará de representar un valor sobreestimado.

Relativo a la liberación de Bisfenol A en biberones de policarbonato

Sexta:

Algunos detergentes, en contacto con biberones de policarbonato, promueven la degradación de este polímero y, como consecuencia, incrementan la liberación de bisfenol A que, posteriormente, podrá ser ingerido con el alimento. El grado de degradación va a depender del detergente utilizado y la liberación en algunos casos sigue teniendo lugar incluso después de haber eliminado el detergente de la superficie del polímero.

Séptima:

Algunas aminas son responsables de un mayor grado degradación del policarbonato. De las 16 sustancias estudiadas, 4 claramente inducen la liberación de bisfenol A y, de éstas, las que se distinguieron por un elevado grado de liberación de bisfenol A fueron dos diaminas alifáticas de bajo peso molecular. También se demostró que la liberación de bisfenol A se debe efectivamente a la presencia de la amina en disolución y no debido al aumento del pH provocado por la misma.